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TABLE OF CONTENTS

VOLUME 59 APRIL 1955 NUMBER 4

ORIGINAL ARTICLES

PAGE

Pathology of Cerebral Palsy
Abe Towbin, M.D., Columbus, Ohio 397

Granulomatous Thyroiditis with Anisotropic Crystalline Material
Stanley Gross, M.D., New York 412

Atherosclerosis
Edwin F. Hirsch, M.D., and Richard Nailor, Ph.D., Chicago 419

Responses of the Liver to Injury
Joseph Hoffman, M.D.; Marion B. Himes, Ph.D.; Sybil Lapan, B.A.; Rose Riszki, M.A., and Joseph Post, M.D., New York 429

Fine Structure of Renal Glomerulus as Revealed by Electron Microscopy
James F. Rinehart, M.D., San Francisco 439

Multiple Arteriosclerotic Aneurysms of Coronary Arteries
William E. Loring, M.D., Chapel Hill, N. C. 449

Lipogenesis by Cells of Cornea
David G. Cogan, M.D., and Toichiro Kuwabara, M.D., Boston 453

Polyostotic Fibrous Dysplasia of Bone
John V. Denko, M.D., Amarillo, Texas, and Theodore L. Perrin, M.D., Seattle 457

Anomalous Connection of Pulmonary Veins with Normal Pulmonary Venous Drainage
Luis M. Becu, M.D.; Newlon Tauxe, M.D.; James W. DuShane, M.D., and Jesse E. Edwards, M.D., Rochester, Minn. 463

Radioautographic Localization of Na^{22} in the Rat Kidney
Jacob S. Krakusin, M.D., Ph.D., Dallas, Texas, and Robert B. Jennings, M.D., Chicago 471

Studies on the Viscosity of the Cytoplasm of Mouse Ehrlich Ascites Tumor Cells
Edwin T. Nishimura, M.D.; Joseph A. DiPaolo, Ph.D., and Willard T. Hill, M.D., Chicago 487

Pathologic Effects of Intravenously Administered Pyrogens on Large Animals
John H. Rust, D.V.M., Chicago; Sam L. Hood, Ph.D., Yellow Springs, Ohio, and C. L. Comar, Ph.D., Oak Ridge, Tenn. 495

SCIENTIFIC EXHIBITS

Neoplasms of the Thyroid
John B. Hazard, M.D.; George Crile Jr., M.D.; Robert S. Dinsmore, M.D.; W. A. Hawk, M.D., and Rex Kenyon, M.D., Cleveland 502

REGULAR DEPARTMENTS

News and Comment 418, 462, 501

Books 514

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VOLUME 59 APRIL 1955 NUMBER 4

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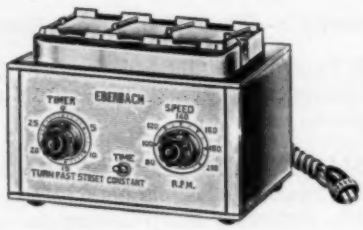

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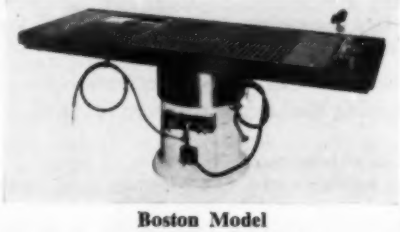

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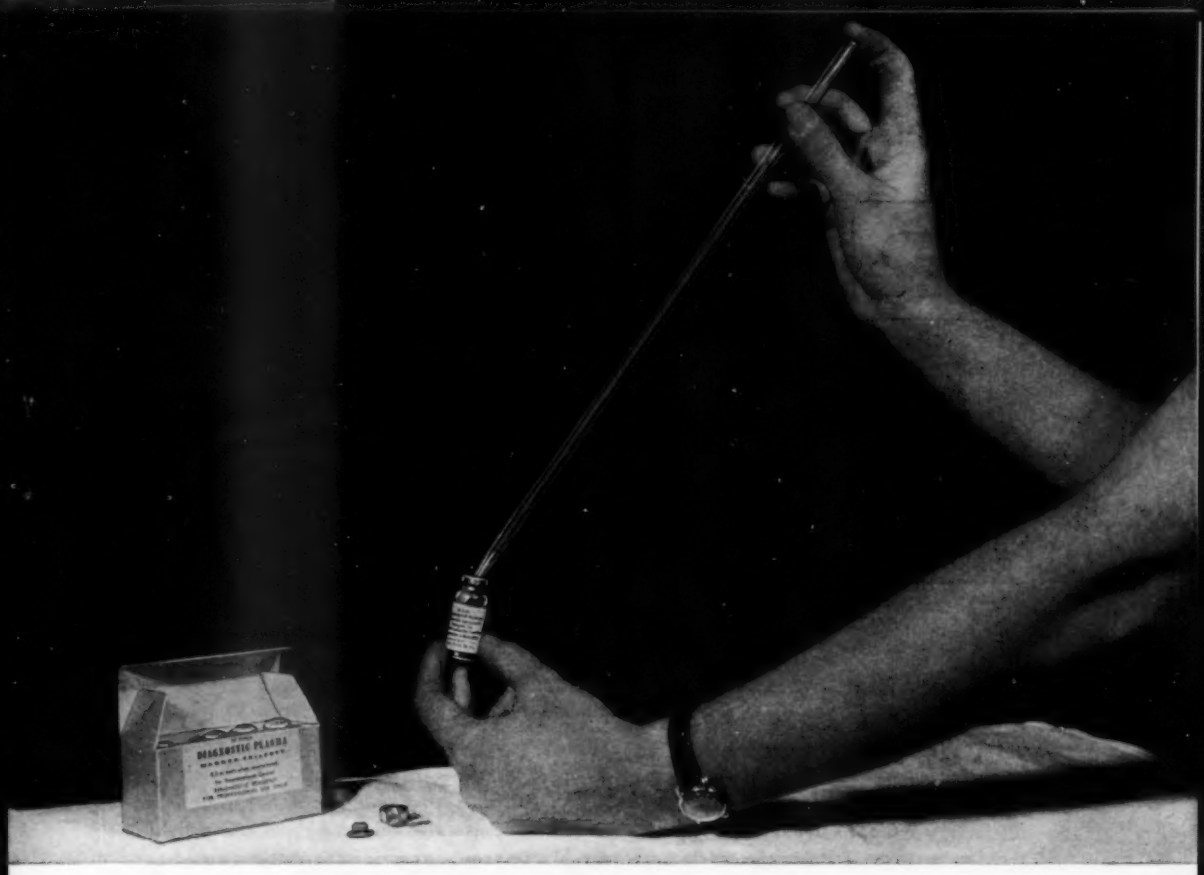

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Pathology of Cerebral Palsy

I. Developmental Defects of the Brain as a Cause of Cerebral Palsy

ABE TOWBIN, M.D., Columbus, Ohio

Over 300,000 cases of cerebral palsy are estimated to be present in the population of the United States today. The occurrence of this disorder occupies increasingly the attention of both laymen and physicians. The clinical aspects of cerebral palsy, diagnosis, treatment, and habilitation, have been the subject of wide discussion and investigation. However, in sharp contrast, much less critical attention has been given to the study of the underlying pathologic processes in this disorder. Knowledge regarding the essential nature of cerebral palsy remains obscure; there exists a general unawareness of the etiology and organic changes in this disorder. Medical thinking, long wedded to the theory that cerebral palsy is due to mechanical head injury incurred at birth, has failed to keep pace with recent investigative findings. Laymen, indoctrinated by the press, television, and other media, have likewise absorbed the conception that cerebral palsy is the result of direct injury suffered by the newborn infant as it is extracted from the birth canal; commonly blamed is the crushing of the head by the obstetrician's forceps.

To the pathologist the analysis of cases of cerebral palsy has long presented a challenge; there has been wide disagreement in the interpretation of the nature of this disorder. The study of the pathologic processes in cerebral palsy merits wider consideration. In recent decades investigations have been carried on in many clinical provinces, in neurology, obstetrics, pediatrics, and hematology. The results of these studies indicate that many mechanisms, often appearing unrelated, contribute to the occurrence of this multifaceted disease. As the mosaic of etiologic factors is fitted together, the complexities of cerebral palsy are gradually resolved.

The pathology of cerebral palsy has been the subject of much confusion and misinformation. In 1841, in the original description of the disease that bears his name, Little[1] postulated that cerebral palsy was probably due to a diffuse involvement of the brain; some parts of the brain, he noted, were apparently more vulnerable than others. He observed that prolonged birth often anteceded the development of the disease; he suggested that asphyxia might be an etiologic factor.

Notwithstanding this deduction by Little, there emerged in the latter part of the 19th century the concept that cerebral palsy was an entity resulting from cranial trauma, associated with meningeal hemorrhage, incurred at birth. Recent discoveries defining other mechanisms of brain damage in cerebral palsy have been little able to modify this firmly cemented misconception.

The singular incident that did much to link meningeal hemorrhage and cerebral palsy is worthy of note. In 1885 McNutt,* presenting a thesis on the subject of infantile cerebral palsy, analyzed, first, the case of a 2½-year-old child who had been delivered instrumentally and who portrayed the typical clinical pattern of bilateral spastic palsy; at autopsy the brain in this case showed bilateral walnut-kernel distortion of the convolutions in the motor area. McNutt correlated this autopsy with a case of left-sided hemiplegia in a 28-day-old infant who at autopsy showed

The Columbus State School and the Department of Pathology, Ohio State University College of Medicine.

* References 2 and 3.

"the right hemisphere covered by a clot which had broken up the central convolutions, the optic thalamus, and the corpus striatum."

McNutt associated the autopsy findings in this case with the etiology of cerebral palsy; in her appraisal of this case she stated: "it is a legitimate conclusion that had this case lived, it would have developed by cicatrization the shrinkage of the central convolutions, and possibly the sinus into the lateral ventricle with the convergence of the neighboring convolutions, which characterize the most marked cases of this disease." Regarding McNutt's deduction, Collier,[4] writing in 1924, notes that "it introduced a glaring error which has remained until this day, and which, although pathologically untenable and resting upon no evidence whatsoever, seems to have defied all the attempts which writers in the past 25 years have made to eradicate it."

Many investigators have pursued this deviative problem. Osler,[5] Gowers,[6] and other notable students of neurology lent support to the theory of McNutt. Freud,[7] on the other hand, greatly interested in the problem of cerebral palsy early in his career, rejected the hypothesis that direct injury was the cause of cerebral palsy; he toured the pathology laboratories and museums of Europe and searched the literature. Concluding this investigation Freud stated that, although cases of infantile hemiplegia could be attributed to cranial injury and meningeal hemorrhage, in no instance was he able to identify direct mechanical trauma as a confirmed cause of the common diplegic form of cerebral palsy; he believed that spastic diplegia was largely due to developmental disturbances.

DEFINITION OF CEREBRAL PALSY

The term cerebral palsy refers to a group of neurological disorders having certain features in common (Table 1). A wide range of neurological disturbances are included under the designation of cerebral palsy. The inconstancy in the causes and clinical patterns of cerebral palsy tends to deter consideration of this disorder as a medical entity. The problem here is similar to that encountered in the study of certain other pathologic processes. For example, in considering cirrhosis of the liver, although the various types possess obvious differences in etiology and pattern, there are fundamental relationships in the clinical, pathological, and therapeutic aspects of the various forms of this disease; accordingly, cirrhosis warrants consideration as a medical entity. Likewise, an appraisal of the various forms of cerebral palsy reveals many clinical and pathologic factors which link this group of neurologic disorders.

In defining cerebral palsy (Table 1), the time of incurrence of the disease is a distinctive criterion. The brain lesion in cerebral palsy is incurred during intrauterine life or, in some instances, in early infancy.

TABLE. 1.—*Definition of Cerebral Palsy*

Time of incurrence of lesion	During embryonic, fetal, or early infantile life
Location of lesion in the brain	Pyramidal tract; extrapyramidal system; occasionally cerebellar
Clinical features	Spastic (approximately 40% of cases)
	Athetoid (approximately 40% of cases)
	Ataxic and other types (approximately 20% of cases)
Commonly associated disorders	Mental retardation (severe mental defect, 30% of cases), convulsive disorders
Etiologic types	Developmental: Hereditary or induced arrest of cerebral development
	Systemic: Encephaloclastic lesions resulting from systemic disorders of the fetus or newborn
	Local: Destructive cerebral processes originating intracranially

The second distinctive feature in cerebral palsy concerns the location of the lesion (Table 1). Cerebral palsy is in most cases a disorder of the pyramidal or extrapyramidal system of the brain; disturbances which are primarily of the spinal cord or of the peripheral nerves are to be excluded. Irrespective of the direct cause of the lesion, the clinical patterns portrayed by patients with cerebral palsy tend to have basic similarities due to the common location of the lesions in the brain. Often involvement of the motor system is combined with lesions in other parts of the brain; in such complexes, crippling of mental and sensory facilities may be manifested.

Clinically, the most familiar neurological pattern of cerebral palsy presents varying degrees of spasticity of the extremities due to upper motor neuron involvement; pathologically, the lesion is usually located in the pyramidal tract at the level of the motor cortex or in the internal capsule. This neurologic pattern comprises 40 to 60% of cases clinically. The term diplegia is applied in defining cerebral palsy in which there is spasticity of severe degree in the lower extremities and of moderate degree in the upper extremities. The wider concept of cerebral palsy includes unilateral and asymmetric disturbances of the motor mechanism such as hemiplegia. The second basic neurological pattern of cerebral palsy is athetosis; this clinical form accounts for approximately 20 to 40% of cases. Motor defects of cerebellar origin make up a small group of cases of cerebral palsy.

Classification of the different forms of cerebral palsy can be based upon various criteria.[8] If the causes of a disease have not been defined, classification may be based on clinical features of the patients during life or on morphologic patterns of the disease as observed at necropsy. In the past, cerebral palsy has usually been classified according to neurologic symptoms, topographical involvement, and other bodily manifestations of the disease; such classifications are valuable in the appraisal of the disease clinically. Etiologic factors, if they are known, provide the most secure foundation in classifying a disease. Only in recent years, as the processes responsible for cerebral palsy have become clarified, has a classification on the basis of etiology become feasible.

The causative agents responsible for the lesions in cerebral palsy are of heterogeneous nature; a large number of etiologic factors have been indicated. During fetal life, the period in which most processes of cerebral palsy are initiated, the brain is exposed and vulnerable to diverse injurious agents, traumatic, metabolic, and infectious. Some of the factors charged with the cause of cerebral palsy are rare; some require further confirmation.

Classification of the pathologic changes in the brain in cerebral palsy has been the subject of much confusion because of ambiguity in terminology. Josephy [9] points out that "in this field many old-time terms and designations are still in use; they are used indiscriminately and without regard to their actual meaning, together with terms which refer to well-defined morbid entities."

In order to remove the problem of etiology from the province of speculation, clinical studies must be carefully integrated with autopsy findings. A comprehensive analysis of the causes of cerebral palsy is not possible without direct examination of the lesions that occur in the brain. Although many aspects of the organic changes in cerebral palsy have been studied in the past, there is need presently for a reevaluation of autopsy findings in the light of recent investigative discoveries. In interpreting the pathologic changes of cerebral palsy, effort must be made to correlate the cerebral lesions with processes such as erythroblastosis fetalis, anoxia, infectious diseases, and other processes whose direct etiologic association with cerebral palsy has but recently been confirmed.

Frequently stressed by clinical investigators is the need for wider autopsy investigation of cerebral palsy by the pathologist.† Despite the wide incidence of cerebral palsy and despite the current interest in this disorder clinically, few studies of cerebral palsy based on broad series of autopsied cases appear in the literature of pathology; this paucity in autopsy data is due to the fact that, in most circumstances, afflicted persons die at home or in sanitaria where autopsies are not done; information regarding the pathologic changes in the nervous system are buried with the remains and lost forever.

In analyzing the pathology of cerebral palsy, it is requisite that the cases studied at autopsy be of late childhood or older, and that the cases represent a relatively severe degree of clinical involvement. In young infants the nervous system is not completely developed; defects in the motor system are

† References 8, 10, and 11.

difficult to evaluate and the diagnosis of cerebral palsy is difficult to confirm. In the very young, correlation of clinical and pathological findings is often inconclusive. In many aspects, definitive studies in cerebral palsy may be carried out more accurately in older patients. However, necropsy studies of older children with severe cerebral palsy are infrequently available; almost without exception, because of serious nutritive disturbances, few infants with severe motor deficiency survive; in most circumstances the dedicated care required for prolonged life is not available.

In appraising the pathology of cerebral palsy, a valuable key to the problem is provided by the study of cases which display pronounced disease clinically and which, correspondingly, present severe, vivid morphologic changes at autopsy. In cerebral palsy, minimal and moderate clinical forms often provide only vague and inconclusive pathologic changes at autopsy ‡; hence, the mechanisms involved in cerebral palsy must be sought in cases with advanced neurological involvement.

Opportunity was available in the present study to analyze at autopsy 23 cases, infants, children, and adults, who during life had presented severe motor defects related to cerebral palsy. The cases comprising this study are mainly from the autopsy series performed by me at Columbus State School, Columbus, Ohio, between 1950 and 1954. Classification of pathologic processes in cerebral palsy, with reference to these cases, forms the basis of this report. Although the classification that follows is necessarily subject to some degree of overlapping, it permits a general panoramic analysis of the principal causes of cerebral palsy.

‡ References 11 and 12.

TYPES OF ETIOLOGIC PROCESSES IN CEREBRAL PALSY

In analyzing the underlying etiologic processes in the present study, three basic types of cerebral palsy were evident (Table 2). Cases could be classified according to the origin and nature of the cerebral defect.

1. *Developmental.*—In this type of cerebral palsy there is present in the brain a developmental defect of hereditary or induced nature. Other forms of cerebral palsy are the result of direct destruction of brain tissue; the developmental type of cerebral palsy is due essentially to a failure in growth and differentiation of the brain.

TABLE 2.—*Etiologic Types of Cerebral Palsy*

Type and Origin	Pathologic Process	Lesions
1. Developmental: Hereditary; induced	Arrest of growth and differentiation	Cerebral hypogenesis and dysgenesis
2. Encephaloclastic; systemic causes	Anoxia neonatorum	Focal necrosis; cystic lesions; status marmoratus
	Prematurity	Hemorrhage, necrosis in the brain
	Erythroblastosis fetalis; systemic septic states	Kernicterus
3. Encephaloclastic; local causes	Mechanical intracranial injury; paranatal and postnatal	Meningeal hemorrhage; contusion and laceration of brain
	Circulatory disorders of the brain; cerebral thrombosis, embolism	Cerebral ischemia and infarction
	Hydrocephalus: Congenital; postinfectious	Damage to motor cortex due to compression and thinning of cerebral wall

2. *Encephaloclastic, Systemic Causes.*—Cerebral palsy of this type results from destructive process in the brain; the lesion in the brain represents a dominant manifestation of a systemic disorder. In the fetus and newborn infant, prolonged anoxia and systemic processes such as erythroblastosis fetalis exert selective damaging effects on the immature brain.[13] The fetus, subjected to such noxae, may belatedly develop encephaloclastic lesions, and, if the infant survives, symptoms of cerebral palsy may be anticipated. In considering the pathology

of cerebral palsy, it is of fundamental importance to distinguish cerebral defects due to malformation from defects due to destruction.§ Birnbaum [15] stresses the point that, in general, malformations originate in the first three months of intrauterine life, whereas the so-called fetal diseases attack the already formed fetus. Yakovlev and Wadsworth [16] also stress the difference between schizencephalic defects, which are the result of a developmental failure of the brain, and encephaloclastic defects, that are a result of frank necrosis late in fetal life.

3. *Encephaloclastic, Local Origin.*—Cerebral palsy of this type is the result of intrinsic disorders of the brain; if the still-unfinished brain of the fetus or infant is damaged by mechanical injury, infarction, or other local encephaloclastic processes, cerebral palsy may ultimately be manifested.

With reference to their inception, the three types of cerebral palsy have a distinct temporal relationship: The developmental type usually has its genesis in embryonic or early fetal life; the second type of cerebral palsy, that resulting from erythroblastosis fetalis, anoxia, and other systemic disorders of the fetus, is generally initiated in the intermediate or late stages of fetal life; the third type of cerebral palsy, that which follows in the wake of local encephaloclastic processes, is usually incurred in late fetal or early infantile life.

In the study of cerebral palsy as in the consideration of other organic disorders, it is important to distinguish processes which originate locally from processes which are a part of a systemic disease. This division is of particular significance in the nervous system; for example, in the clinical evaluation of a patient with cord symptoms, it is of immediate importance to determine whether the involvement represents a local lesion of the nervous system or a secondary manifestation of a systemic disorder such as pernicious anemia. Likewise, in some instances cerebral palsy originates as a primary local defect of the brain; in other cases it represents the residual nervous system component of a bodily disorder; parenthetically, in the treatment and prophylaxis of cerebral palsy these factors must be given early consideration. In recent decades there has been an increased effort in neuropathology to recognize and delineate disorders of the brain which are related to generalized organic disorders of the body. With cerebral palsy considered in this light, the attention of pathologists must be focused more and more on the forms of this disorder arising systemically.

The present study will be presented in two parts: The first will deal with cerebral palsy due to developmental defects of the brain; the second, to be reported later, will deal with the types of cerebral palsy which are due to encephaloclastic processes.

§ References 9 and 14.

DEVELOPMENTAL DEFECTS AS A CAUSE OF CEREBRAL PALSY

In appraising the causes of cerebral palsy, it is evident that in one group the defect in the brain is due to a faulty organogenesis, an imperfection in the development of the brain (Table 1).‖ Cases of spastic diplegia related to agenesis of the cerebral wall are described by Yakovlev and Wadsworth.[16] Malformation of the brain was evident in approximately 30% of cases of cerebral palsy studied at autopsy by Norman.

Defective or arrested development may come about in two ways: 1. The anomaly of the brain may be due to a transmitted Mendelian hereditary genetic factor; the original germ plasm forming the central nervous system may be defective or inadequate. Clinically, in this form of developmental defect, multiple incidence of the anomaly may be observed in a family or antecedents. 2. The developmental defect in the brain may be induced. In such instances, the original germ plasm is intact, however, during embryonic or fetal life an illness or injury in the mother subjects the fetus to unfavorable intrauterine influences. This period of gestational stress, imprinted on the fetal nervous system in the form of a developmental defect, often proves to be the prelude to cerebral palsy.

‖ References 12, 17, and 18.

Although the pathogenesis of hereditary and induced developmental anomalies is different, separation of the two forms on a morphologic basis is difficult. The induced form of cerebral defects, contracted during early gestation and analyzed after birth, often cannot be distinguished with certainty from primary hereditary anomalies. Clinical investigation of the patient's antecedents may divulge evidence of a hereditary basis of the cerebral disorder. A distinct history of maternal illness during pregnancy aids in identifying induced developmental defects; in infants harboring a developmental arrest of the brain as a consequence of gestational stress, the presence of synchronous congenital anomalies in the heart and other sites tends to confirm the induced nature of the cerebral defect. Collier [4] believed that cerebral diplegia was essentially due to an arrest in neuronal development due to noxious influences of unknown nature and that only seldom did the disorder appear to be hereditary; he cites rare instances in which a diplegic mother gave birth to a child similarly affected. Davel [17] notes that very ocasionally it happens that identical cases of cerebral palsy appear in one family or successive generations. Yannet [18] likewise presents evidence suggesting that a genetic factor may be responsible for cerebral palsy in some cases.

Clinical studies indicate, however, that in most cases of cerebral palsy associated with defective development the brain anomaly is induced rather than hereditary. Evans,[19] in a clinical study of 114 cases of cerebral palsy, found no instance of cerebral palsy in grandparents, parents, uncles, or aunts of the patients; one microcephalic hemiplegic boy had a microcephalic tetraplegic brother.

Morphologically, developmental arrest of the brain in cases of cerebral palsy varies in degree. Agenesis, the extreme developmental failure of the brain, exemplified by anencephaly, is incompatible with life. Developmental arrest in infants that survive is of incomplete degree, hypogenesis. The entire organ may be blighted or only a circumscribed part of the organ may show developmental failure. If the brain is involved *in toto*, microcephaly is manifested. Little,[20] in 1862, in his description of the disorder called attention to the common occurrence of smallness of the head in infants afflicted with spasticity. Other observers ¶ point out that microcephaly is characteristic of patients with spastic diplegia, irrespective of the extent of neurologic involvement. Microcephaly vera refers to a brain small because of a failure in growth and development; cerebral palsy in these instances is largely attributable to the undifferentiated state of the motor cortex. True microcephaly is to be distinguished from pseudomicrocephaly, in which the brain is small and distorted because of destructive processes which have reduced the size of the organ.

True microcephaly presents two basic characteristics: arrest in differentiation and smallness. Failure in maturation, the retention of a fetal architecture for years after birth, may be a more striking gross feature than smallness; the brain structure in such cases recapitulates that of a lower animal; this manner of defect is exemplified in Cases 1 and 2 of this study. In other instances of microcephaly vera the most obvious feature is smallness; the parts of the brain appear relatively mature and proportionate, but the brain is Lilliputian; it will be seen that the brain in Case 6 is of this nature. Close examination of such brains reveals gross as well as microscopic underdevelopment.

Microcephaly is commonly an induced developmental defect. This has been confirmed experimentally in laboratory animals and clinically in man. In some cases, however, microcephaly may represent a true hereditary anomaly; microcephaly of this nature is observed in groups of Australian aborigines. Sporadic familial incidence of microcephaly occurs throughout the world; reports in the literature describe families in which parents were cousins and one or more children bore the "pinhead" characteristic. In hereditary microcephaly, the brain, although in miniature, is usually well differentiated.[21]

¶ References 13 and 18.

Arrested development may be limited to a lobe or other localized portion of the brain. Such regional hypogenesis is generally more difficult to discern than microcephaly; localized developmental stigmas tend to be symmetrical. Developmental malformations may take the form of overgrowths. In other instances normal fusion of parts may fail, or anatomic relationships in the brain may be misarranged. In the cerebrum, gyral anomalies are common; the gyral pattern may be irregular; there may be agyria, microgyria, or macrogyria.

Histologic confirmation of developmental cerebral arrest is frequently available in the form of neuronal heterotopia. Normally in the differentiation of the cerebrum neuronal elements in the mantle layer migrate outward to form the gray layer of the cortex. This migration may prove incomplete. Neurons, lagging behind, may be identified in the cortical white matter even in adulthood. The presence of such heterotopic deposits is a distinct measure of developmental arrest.

Although it is sometimes difficult in the adult brain to distinguish with certainty defects due to arrested fetal development from lesions due to remote deterioration of once intact brain tissue, effort should be made to identify this difference. Evidence of both developmental and encephaloclastic defects may be present in the same case. Examined after birth, a lesion attributed to a developmental fault is free of gross and histologic signs of frank necrosis and shows no evidence of true atrophy. Injuries incurred by the fetal brain late in gestation usually leave characteristic telltale marks and can generally be correctly identified after birth. The residues of destructive fetal processes may assume the form of cortical cysts, foci of necrosis, and areas of gliosis and atrophy.

In the study of cerebral palsy a consideration of the mechanisms responsible for induced developmental defects is of imminent importance; this is particularly significant in the prophylaxis of cerebral palsy. The agents and processes involved in the production of developmental defects have been the subject of wide investigation. Gruenwald [22] points out that abnormal development can be induced experimentally in the embryo by any number of physical and chemical agents. In the human the brain appears to be the part most susceptible to induced developmental defects and is the most commonly affected. Murphy [23] notes that approximately 60% of malformations primarily involve the nervous system.

Two processes, occurring early in pregnancy, are known clinically to induce developmental defects in the brain in the human: maternal rubella # and maternal irradiation.* Many cases of cerebral palsy have been directly attributed to maternal assault by these processes. Other factors, such as anoxia,† dietary deficiency,[30] toxic agents,[28] psychotic states [15] in the early months of pregnancy, have also been charged with the cause of developmental defects of the nervous system.

Infectious disease occurring early in pregnancy, long suspected as a factor contributing to the induction of developmental defects in offspring, has in recent decades been confirmed clinically as a direct cause. Rubella infection in the mother during the first or second month of pregnancy provokes fetal malformations in nearly 100% of cases.[22] The nervous system is particularly vulnerable; growth and differentiation of the fetal brain frequently is stunted after maternal infection with rubella. In children with antecedent intrauterine exposure, mental retardation, motor defects, and other central nervous system disturbances are reported.‡ Swan and co-workers § reported that microcephaly developed in 11 of 49 pregnancies complicated by rubella in the first trimester. Cerebral palsy appearing in microcephalic persons of this nature can be attributed to the antecedent maternal infection. The mechanism by which maternal rubella exerts its influence on fetal development is a matter of question. Whether the fetal tissues are actually infected or whether the defective development

References 24 through 26.
* References 23 and 27.
† References 28 and 29.
‡ References 24 through 26.
§ References 24 and 31.

is the result of disturbed intrauterine environment has not been established. Experimental studies indicate that the human chorionic epithelium is by nature generally resistant to viruses [32]; hence, direct transplacental infection appears unlikely.

Mumps and other maternal infectious processes have on occasion been considered responsible for the occurrence of developmental defects in the brain. Worcester and co-workers [33] found that a high incidence of malformations occur when the first trimester of pregnancy occupies the fourth or first quarters of the year, a time when respiratory diseases are common. Syphilis does not induce developmental faults in the early fetus [23]; this is borne out by the fact that an infected mother treated before the fifth month of pregnancy will usually give birth to a normally developed child; however, cerebral palsy may appear in the offspring of untreated syphilitic mothers as a result of destructive cerebral lesions incurred late in fetal life.

Irradiation during early pregnancy regularly induces developmental malformations in offspring [23]; this is evident clinically and has been confirmed experimentally. Hicks [34] has demonstrated experimentally the high susceptibility of the fetal nervous system to irradiation and to certain radiomimetic agents such as nitrogen mustard and aminopterin. Hicks postulates that ionizing radiation and the radiomimetic agents affect developmental arrest by inhibiting enzyme action in the cells of anlage tissue. Clinically, irradiation is known to induce nervous system anomalies in the human; Murphy [23] and others have reported the occurrence of microcephalic offspring following pelvic irradiation of the mother during the early months of pregnancy. Murphy found that in 75 women who received therapeutic amounts of pelvic irradiation in early pregnancy, 17 cases of microcephaly occurred in the offspring. Plummer,[27] in a study of anomalies occurring in children exposed in utero early in pregnancy to the atomic bomb in Hiroshima, reported that 7 out of 11 mothers within 1200 meters of the hypocenter were delivered of microcephalic mentally retarded children.

Anoxia is known to induce developmental defects in the lower forms of animal life. The effects of anoxia on early fetal brain in the mammal is not definite.[22] In clinical studies, Murphy [23] and Worcester and co-workers [33] have recognized a relatively high incidence of malformations in the offspring of anemic mothers. Some investigators have suggested that the underlying mechanism for all induced developmental defects may be a derangement of metabolism and that the common denominator in all such processes may be anoxia. Anoxia late in gestation or at birth causes severe destructive changes in the late fetal brain; as will be evident later, this encephaloclastic effect of systemic anoxia, occurring in the older fetus, is a major cause of cerebral palsy. Although Cook [29] suggests that prolonged oxygen deficiency in the first weeks of gestation might result in permanent retardation of brain development, this mechanism has not been demonstrated to be a significant cause of cerebral palsy.

Mental stress in pregnant mothers as a cause of developmental defects in offspring has long been a subject of speculation. The possibility exists, notes Birnbaum,[15] that emotional distress during pregnancy may result in interference with the process of fetal development. In the offspring of mothers who have been subjected to concentration camp internment in Germany, a high incidence of developmental defects was observed [21]; hydrocephalus, anencephaly, and Mongolism occurred in a percentage many times above the normal expectation. Dietary deficiency during pregnancy has also been implicated as a cause of developmental anomalies; riboflavin deficiency in experimental animals has been shown to induce developmental defects.[30]

Toxic states have sometimes been charged with the cause of induced developmental defects; however, toxemia of pregnancy and maternal hypertension do not appear to be responsible factors.|| Exogenous poisons have been related to the induction of develop-

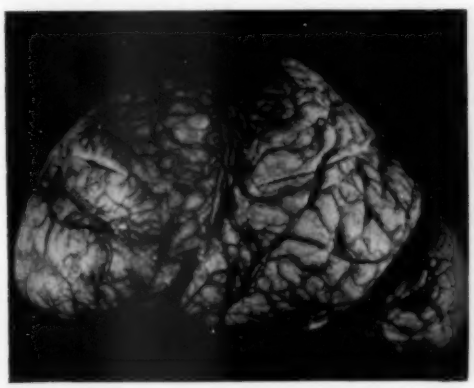
|| References 23 and 33.

Fig. 1 (Case 1).—Developmental arrest of the brain; microcephaly vera. Brain has fetal architectural pattern. Patient was 8 years old; spasticity and mental arrest were noted early in infancy. Brain weight 800 gm. Superior view.

mental malformations experimentally; a high incidence of malformation is said to occur in the offspring of mothers with chronic lead poisoning.[15]

Cerebral palsy due to developmental defects of the brain is in some aspects closely linked to the group that results from encephaloclasia in late fetal life. The mechanism responsible for the induction of developmental defects of the brain in the early fetus is related to the systemic processes which produce direct damage to the brain in the maturing fetus and newborn infant. Although the final organic changes portrayed by these two types are distinctive, there are, nevertheless, certain etiologic factors common to both processes. In both types of cerebral palsy the brain defects may result from the same basic forms of gestational stress. The difference lies in the temporal element. As a result of stress occurring early in fetal life, the anlage tissue of the brain is molded imperfectly; developmental defects of the brain are the consequence; occurring late in gestation, fetal stress provokes frank cerebral destruction. Because of the close causal relationship of these two types of cerebral palsy, classification is complicated by some degree of overlapping. However, the wide difference in the pathologic processes, inhibition of growth in one type and destruction of maturing brain tissue in the other, renders necessary a separate consideration of these types of cerebral palsy.

Fig. 2 (Case 1).—Lateral view.



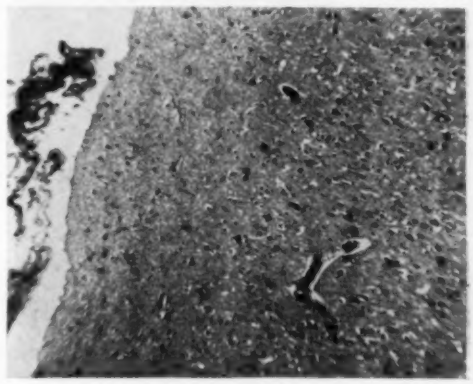


Fig. 3 (Case 1).—Histologic pattern of motor cortex. Lamellar pattern is poorly defined; large and small nerve cells are scattered haphazardly through the cortex. Hematoxylin and eosin; reduced 3/7 from mag. × 140.

In the present investigation, six cases with developmental cerebral defects showing varying degrees of motor and mental insufficiency clinically were available for study at autopsy. Motor disturbances during life could be correlated with a corresponding degree of morphologic imperfection at necropsy. In this group, cases which showed serious motor handicaps, clinically, at autopsy generally revealed a profound degree of undifferentiation of the brain. The following six cases demonstrate cerebral palsy of the developmental type; the cerebral impairment in the first case of this group is severest; the level

of involvement in the other cases is generally of successively less degree.

REPORT OF CASES

CASE 1 (Autopsy 10295).—Age 8 years, white boy; microcephaly; spasticity; athetosis; associated severe mental retardation and sensory defects.

No history of maternal illness was defined in this case; delivery occurred at term. There was no hereditary trait of mental disease. Spasticity and arrested mental development were evident at an early age. The infant failed to nurse properly. He continued to be helpless throughout life. Head circumference was 17½ in. (44.5 cm.). Neurological examination revealed hyperactive reflexes throughout. The upper extremities were held in flexion at the elbows, the lower extremities extended.

Fig. 4 (Case 2).—Developmental arrest of the brain; microcephaly vera. Macrogyri in the motor cortex. Clinically, this 10-month-old infant had irregular muscular twitchings and showed little motor control. Brain weighed 750 gm. Superior view.

The brain, at autopsy, weighed 800 gm. The outstanding gross feature was the smooth surface of the cerebrum (Figs. 1 and 2); sulci were few and abortive. The small smooth brain resembled very much the organ of a 5-month fetus. Malformation of other organs was not present. The lamellar pattern of the cerebral cortex was ill-defined (Fig. 3). Large and small neurons were scattered haphazardly through the cortex.

This case illustrates an example of extreme developmental cerebral arrest; the involvement of the brain was uniform; spasticity and other severe motor defects were associated with a correspondingly low level of mental development. Clinically, the severe mental and neurologic simplification present in patients such as this tends to overshadow the symptoms of spasticity and other motor disturbances present. This type of microcephaly, however, demonstrates a basic etiologic mechanism in cerebral palsy, that of developmental failure.

Fig. 5 (Case 2).—Transection of brain. Cerebral wall is thick; aborted gyral development.

CASE 2 (Autopsy 5372).—Age 10 months, white male infant; microcephaly; motor incoordination; arrested mental development; convulsive state.

This infant, one of twins, was delivered at term. There were no known complications during gestation. The child nursed poorly and was listless. During the 10 months of life he showed little muscle control; he developed muscular twitchings and, later, convulsions.

At autopsy the brain weighed 750 gm. Sulcation was poorly developed. The cerebrum in this case, although irregularly involved, generally resembled that of a seven-month fetus. Bulging macrogyri were present in the motor area of the cerebrum (Fig. 4). On section, the cerebral cortex was thick (Fig. 5). Histologically, the lamellar pattern in

Fig. 6 (Case 2).—Histologic pattern of motor cortex; lamellar pattern poorly organized. Nissl; reduced ¾ from mag. × 100.

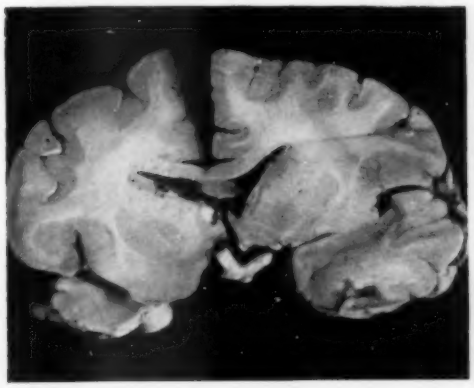
Fig. 7 (Case 3).—Developmental irregularity in convolutional pattern of the cerebrum. Fourteen years old. Spasticity in all extremities. Microcephaly. Brain weight 850 gm. Lateral view.

the motor cortex was poorly oriented; the nerve cells were distributed in a disorderly fashion (Fig. 6).

Hypogenesis in this case was severe but of less degree than the fetalism of the previous case. The differentiation defect in the present case was symmetrical but did not involve the brain uniformly; the motor cortex was more prominently affected than were other portions of the cerebrum. Microcephaly, in the two cases above, reflects a failure in the growth and differentiation of the organ; many microcephalic persons are diplegic.[21]

Case 3 (Autopsy 10481).—Age 14 years, white boy; spastic diplegia; microcephaly; severe mental retardation.

Fig. 8 (Case 3).—Transection through brain. Convolutions irregular and simplified.



This child was one of twins; data regarding gestation and delivery were not available. No hereditary mental disease was known. Evidence of retarded mental and motor development was evident in infancy. The twin of this child was reported to have displayed similar neurologic defects; two other siblings were well mentally. Examination at the time of admission, at the age of 7 years, revealed spasticity of all extremities, particularly the lower. The head circumference was 18 in. (45.7 cm.). The neck was held in opisthotonos. Deep reflexes were hyperactive. The physical examination revealed undescended testes and general constitutional underdevelopment.

The brain at autopsy weighed 850 gm. The cerebrum was disproportionately small; the cerebellum was large and, in relation to the occipital lobe, lay posteriorly rather than inferiorly, resembling the relationship of lower phylogenetic

Fig. 9 (Case 3).—Histologic pattern of motor cortex; lamellar pattern indistinct. Neurons are sparse and irregularly distributed. Hematoxylin and eosin; reduced 3/7 from mag. × 100.

animals. There was considerable irregularity in size and pattern of the cerebral convolutions, particularly in the motor cortex of the cerebrum; this is apparent in the lateral view of the brain, in Figure 7; some convolutions were exaggerated and coarse, others were small and indistinct. This irregularity in convolutional pattern and presence of macrogyria is evident in the surface of the transected cerebrum (Fig. 8); the development of secondary sulci is inadequate. Histologically, the lamellar pattern of the cortex was distorted; neurons were sparsely and irregularly distributed (Fig. 9.). Myelin sheath stains showed poor delineation of the margin between the gray and white matter. The white matter showed ectopic deposits of neurons.

The developmental defect of the nervous system in this 14-year-old patient and, ap-

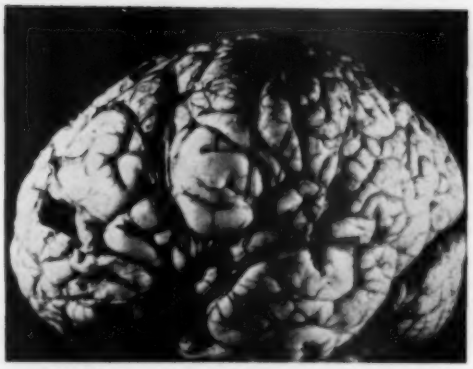


Fig. 10 (Case 4).—Developmental defect of cerebral cortex; convolutional irregularity. Thirty-seven-year-old man with Mongolism and severe muscular incoordination. Brain weight 1300 gm.

parently, in his twin was probably of the induced type and was probably related to a defective twinning mechanism. It will be recalled that twinning was also present in Case 2. Cerebral palsy is relatively common in association with twinning; it may be that defective placentation, inadequate to support twins, provided a gestational state of stress which was reflected as faulty organogenesis in the fetus. Arrested differentiation of the cerebrum is confirmed in this case, histologically, by the presence of neuronal heterotopia, straggling nerve cells in the subcortical white matter.

Case 4 (Autopsy 10710).—Age 37 years, white man; mental retardation; Mongolism; muscular incoordination.

Birth occurred at full term. The mother was said to have been under considerable mental strain during the period of pregnancy. Delivery was recorded as "difficult." The patient displayed a Mongoloid facies. Throughout life he showed crude muscular coordination; he had poor control of his upper extremities. The patient frequently made facial grimaces; he was unable to talk.

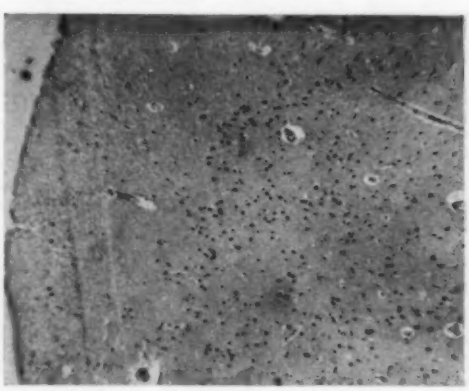
At autopsy the brain weighed 1300 gm. Irregularity of the convolutional pattern of the cerebrum was evident, particularly in the motor areas on each side. Simplification of the convolutions over the inferior aspect of the frontal lobes in this case was also noticeable.

The disordered cerebral tectogenesis in this case resembled that of Case 3 but was not so diffuse. Histologically, the involved cortex in the motor areas showed poor lamellar pattern (Fig. 11).

The occurrence of cerebral palsy in Mongoloids is occasionally observed. Mongolism is aptly designated as the state of the "unfinished" or "ill-finished" child. Mongols represent a deficiency in development which blights the entire body. It is generally thought that Mongolism is induced during embryologic and fetal life owing to inadequate function of the uterus in supplying nutriment and other needs of the fetus; this inadequacy of the uterus may be due to excessive age of the mother, to antecedent disease or injury of the uterus, or to maternal illness during gestation.

In Mongolism, malformations of skeletal structures and organs make their appearance through the body; anomalies of the heart are common; such defects represent developmental defects of the induced type. In the brain, hypoplasia of the cerebellum, pons, and medulla is frequent in Mongolism. Convolutional simplification over the orbital surface of the frontal lobe and other parts of the cerebrum is sometimes apparent. No consistent change is depicted by the motor cortex of the brain in Mongolism. The involvement of the cerebral cortex that occurred in the present case may be correlated with the motor disabilities evident clinically; the findings in this case of Mongolism represent a developmental type of cerebral palsy apparently induced in the fetus as a consequence of gestational stress.

Fig. 11 (Case 4).—Histologic pattern of motor cortex; poor lamellar pattern; irregular distribution of neurons. Reduced 3/7 from mag. × 80.



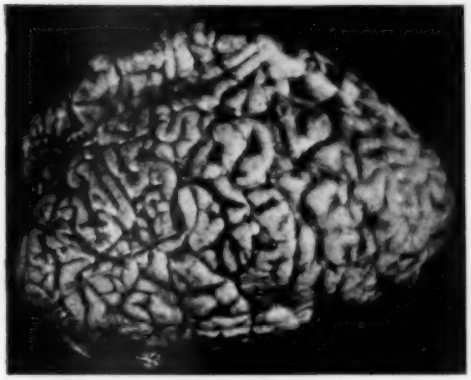


Fig. 12 (Case 5).— Developmental disorder of cerebral cortex; thickened irregular convolutions in the frontal lobe. Thirteen years old; spastic quadriplegia.

CASE 5 (Autopsy 10424).—Age 13 years, white girl; spastic quadriplegia; mental retardation.

Delivery by breech presentation had occurred at term. No maternal illness during pregnancy was recorded. At 6 months of age, it was noticed that the infant showed a lack of response. The patient developed slowly; she was always mute. Extremities were spastic; the patient wore leg braces; she made irregular movements with the legs at times. Deep reflexes were hyperactive.

At autopsy the body showed considerable constitutional underdevelopment. The brain weighed 1250 gm.; the brain was large. The convolutions over the frontal lobes were enlarged and irregular (Fig. 12); the arachnoid over the frontal area was slightly opaque. On section, the white matter in the frontal lobes was relatively decreased in amount. Histologically, the lamellar pattern in the frontal lobes was irregular; the nerve cell population was relatively decreased.

CASE 6 (Autopsy 10898).—Age 7 years, white boy; microcephaly; spastic diplegia; mental retardation.

Delivery occurred at full term; this was the mother's 13th pregnancy. There were nine living siblings in good health. Delivery of this infant was by Caesarean section; there was severe respiratory distress after birth.

The infant was a feeding problem; he was never able to sit up or perform any useful muscular function.

Physical examination at the age of 6 years revealed an underdeveloped, helplessly spastic child, a complete custodial problem. Height was 38 in. (96.5 cm.); weight was 35 lb. (15.9 kg.). The head was noticeably small and measured 16 in. (40.6 cm.) in circumference. There was severe lordosis and scoliosis. The elbows and wrists were held in acute flexion; the legs were adducted and in extreme extension. The deep reflexes were extremely hyperactive. Mental age was estimated at 2.8 months.

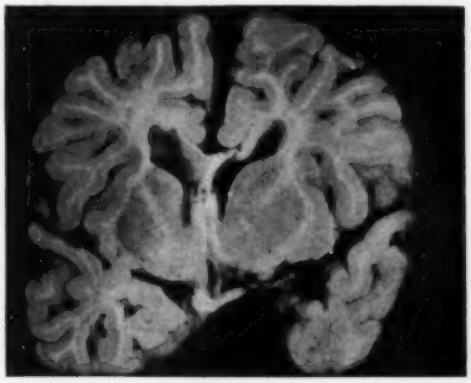
At autopsy, severe constitutional underdevelopment was evident. The brain weighed 640 gm. and appeared approximately one-half average size. The cerebral convolutions were distorted and had a cauliflower pattern. The principal gyri were narrow and deep. On section, the most striking feature in the cerebral hemispheres was the accordion-like appearance of the thin, deep convolutions (Fig. 13); this wizened appearance of the convolutions was exaggerated by the relatively small amount of white matter. The gray matter of the motor cortex was thick; however, histologically, the neurons here were irregularly distributed and in poor lamellar pattern. Myelinization of the corticospinal tracts was deficient in the brain and cord.

It is likely that the state of anoxia at birth accentuated the developmental arrest of the cerebrum and contributed to the motor disturbance in this case. The brain from this 7-year-old child is an example of microcephaly vera, in which the feature of smallness, rather than of gross undifferentiation, is the most striking external feature. The severe hypoplasia of the white matter and the poor differentiation of the neuronal elements in the motor cortex account for the clinical spastic diplegia.

SUMMARY AND CONCLUSIONS

Despite the high incidence of cerebral palsy and the wide clinical interest in this problem, the underlying pathologic processes in this disorder have long remained an enigma. Con-

Fig. 13 (Case 6).—Microcephaly vera. Seven-years old; spastic diplegia. Brain weight 640 gm.; hypoplasia of white matter; deep accordion-like convolutions.



sideration of the etiologic factors and organic changes in cerebral palsy merits wider attention. Little, in his original thesis, postulated that most spastic states observed in children were due to neonatal asphyxia. However, in the last hundred years there gradually evolved the misconception that the dominant etiologic factor in cerebral palsy was related to head injury associated with meningeal hemorrhage incurred during the process of birth. In recent years, investigative discoveries in obstetrics, pediatrics, hematology, and neurology have contributed significantly in dispelling deeply ingrained misconceptions which have attended this problem.

Cerebral palsy refers to motor disturbances which are mainly pyramidal or extrapyramidal in nature. The responsible lesions in the brain vary in origin; all are incurred during intrauterine or early infantile life. In analyzing the cases in the present study, three pathologic types were evident: In the first type the lesion was due to a developmental arrest of the brain. In the second type the cerebral lesion, destructive in nature, was the result of an antecedent systemic disorder present in fetal or infantile life. In the third type, the encephaloclastic lesion represented an intrinsically local intracranial disorder.

The present report deals with the first type, cerebral palsy due to hereditary and induced developmental cerebral defects. Most developmental anomalies of the brain are induced; these defects represent the imprint left on the nervous system by exposure of the early fetus to maternal infectious disease, irradiation, or other noxious gestational influences. Developmental faults of the brain were associated with clinical manifestations of cerebral palsy in six cases of the series studied. In the severest case, an 8-year-old child displaying profound motor disturbances and mental deficiency, the brain at autopsy had the architectural features of a 5-month fetus. In addition to microcephaly vera portrayed grossly, histologic evidence of developmental arrest was evident. In cases of less severe involvement, the brains showed localized anomalous development of the motor cortex. In general, the degree of motor disturbance displayed by the patients during life was proportional to the extent of developmental arrest observed in the brain at autopsy. The findings in this group, constituting approximately one-fourth of the cases of cerebral palsy coming to autopsy during the five-year period of this study, indicated the importance of faulty organogenesis as a cause of cerebral palsy.

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Granulomatous Thyroiditis with Anisotropic Crystalline Material

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Anisotropic crystalline material has been observed and described in sections of human thyroid glands examined with polarized light. Richter * has studied the histologic appearance and frequency of occurrence of such material. Recently these crystals have been identified by the use of the x-ray diffraction method as calcium oxalate monohydrate. Richter studied the general distribution of crystals and found them exclusively in the colloid within thyroid acini. The occurrence and distribution of these crystals in pathologic states was not exhaustively studied.

For the present study, 43 cases of thyroid disease were reviewed. These had been variously classified as chronic thyroiditis, Riedel's struma (struma fibrosa), Hashimoto's struma (struma lymphomatosa), lymphadenoid goiter, pseudotuberculous thyroiditis, and hyperplasia of the thyroid with involution. Among these, 14 cases were characterized by the presence of a granulomatous reaction associated with many so-called pseudotubercles. In sections of all these glands anisotropic crystalline material, morphologically identical with the calcium oxalate crystals described by Richter, was seen when examined with polarized light.

De Quervain described in 1904 [2] a form of nonsuppurative thyroiditis in which one of the prominent histologic features was the presence of tubercle-like cellular conglomerations often associated with giant cells (Fig. 1). Since then this histologic pattern has served to differentiate this group and various names, including pseudotuberculous thyroiditis, giant-cell thyroiditis, and subacute thyroiditis, have been given to it. Still

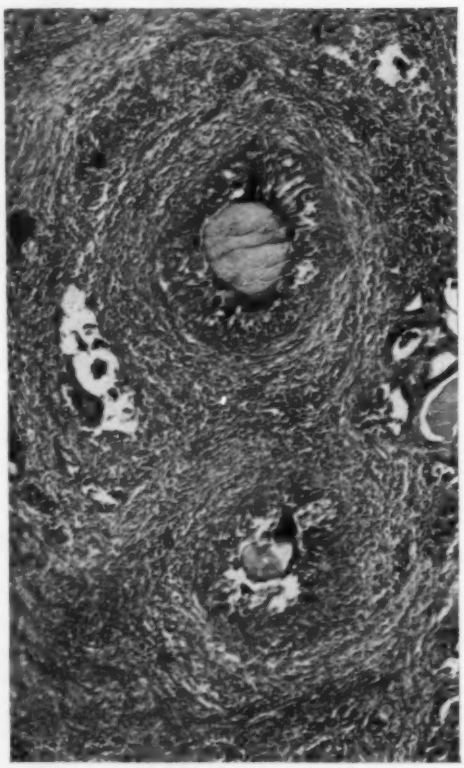


Fig. 1 (Case 6).—Typical granuloma with a central mass of colloid and multinucleated giant cells in the surrounding reaction; reduced ⅓ from mag. × 247.5.

From the Department of Pathology of the College of Medicine and the University Hospital, New York University-Bellevue Medical Center.

* References 1 and 2.

others have referred to such lesions as Riedel's struma. This eponymic designation, I think, is open to serious question, for Riedel's † histologic description was very meager and included mention only of dense fibrous tissue and a "round cell" infiltration.

This report is presented to describe the clinical picture as seen in these 14 patients and to describe the histologic findings, including the occurrence and appearance of the anisotropic crystalline material (calcium oxalate monohydrate).

METHODS

All material studied was fixed in Zenker's fluid (omitting the glacial acetic acid), formalin alcohol, or 10% formalin. (The crystals are dissolved out easily by acids; therefore fixatives containing acids cannot be used.) Sections were cut and stained in the routine fashion with hematoxylin and eosin. Hematoxylin and eosin stained sections were examined with ordinary diffused light and with polarized light for the presence of crystals. "Polaroid" discs were used for the polarization of light but, of course, similar results may be obtained by the use of crossed Nicol prisms.

† References 4 and 5.

CLINICAL OBSERVATIONS

The clinical syndrome presented by these patients conforms to a pattern that has been recognized and described in previous reports (de Quervain and Giordanengo,[6] Crile and Rumsey,[7] Lasser and Grayzel,[8] Lindsay and Dailey [9]). In this series, 13 of the 14 were female patients. The age of onset varied from 30 to 52 years. De Quervain and Giordanengo [6] described nine cases in detail. Eight patients ranged from 39 to 50 years old at the time of onset, while the age was not stated for the ninth. Crile and Rumsey [7] analyzed 38 cases, of which 29 were between 30 and 50 years old and the mean age of onset was 42. The eight cases studied by Lasser and Grayzel [8] varied from 36 to 61 years old. Of the 37 cases presented recently by Lindsay and Dailey [9] 75% were in the fourth and fifth decades, while the range for the entire series was from 23 to 71 years. In all these series, as well as in the present group of cases, there is a very pronounced predilection for the fourth and fifth decades of life.

The onset was often sudden and the history short. Symptoms were present for 2 to 10

Summary—Histories of Fourteen Cases of Granulomatous Thyroiditis

Case	Sex	Age	Duration	Pain and/or Tenderness	Dysphagia	Temperature	Physical Findings of Thyroid	B. M. R. and Cholesterol (Mg./100 Cc.)
1	F	50	10 wk.	0	0	N	Hard, fixed, slightly enlarged	+35%
2	F	44	4 wk.	Present	0	N	Enlarged, firm, tender	.....
3	F	40	1½ yr.	0	0	N	Mass in right lobe	+ 2%
4	F	42	6 wk.	Marked	Present	N	Left lobe enlarged, tender, stony hard	+12%
5	F	47	4 wk.	Present	Present	Up to 101 F	Enlarged	+21% (196)
6	F	36	4 wk.	0	Present	Up to 102 F	Enlarged	+ 9% (180)
7	F	33	2 wk.	Present	0	N	Mass in right lobe	.....
8	F	43	5 wk.	Present	Present	Up to 101 F	Mass in right lobe and isthmus	+ 1%
9	M	56	10 yr.	Present	0	N	Hard, immobile mass on right	—18% (160)
10	F	37	2½ yr.	.....	0	Up to 100 F	Diffuse enlargement	"Normal"
11	F	41	6 wk.	0	0	N	Enlarged, hard	+12%
12	F	30	6 wk.	Present	0	Up to 103 F	Enlarged and tender	.....
13	F	52	4 wk.	Present	0	Up to 102 F	Enlarged and tender	.....
14	F	50	3 wk.	Present	0	Above 100 F	Enlarged, hard, and tender	.....

N indicates no elevation.

Fig. 2 (Case 1).—Anisotropic crystals in a multinucleated giant cell. Compressed acini, some with colloid in surrounding parenchyma. Polarized light with partial extinction; reduced ⅓ from mag. × 620.

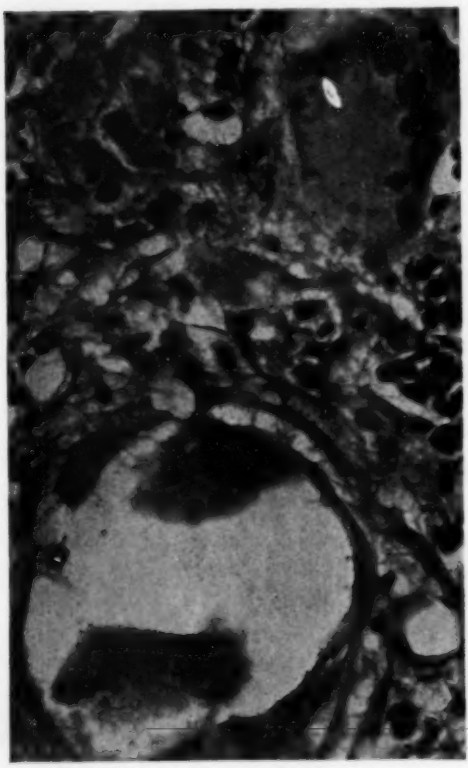
weeks in 11 patients. In two others symptoms were present for 1½ to 2½ years, while in a third case symptoms were present for 10 years, principally because nonsurgical treatment had been tried for a long time.

Pain, usually associated with tenderness, was often a prominent presenting symptom and was present in nine cases. The pain was usually diffuse in the thyroid region, but occasionally was more or less localized to one side. Four patients complained of dysphagia, and in three this was associated with tenderness. Systemic manifestations were evident in some cases. Eight patients had slight to moderate elevations in temperature preoperatively; in one the temperature rose to 103 F. Five patients complained of rapid weight loss; one lost 20 lb. in six weeks following the onset of symptoms.

Physical examination revealed in all cases an enlarged thyroid, sometimes two or three times normal size. The enlargement was often diffuse and at other times nodular. Occasionally the involvement was unilateral and, in a few cases, only a nodule in a portion of one lobe was palpable.

Laboratory examination revealed a mild normochromic anemia in six cases, with the red blood cell counts varying from 3,000,000 to 3,500,000 cells per cu. mm. and 9.6 to 11.3 gm. of hemoglobin per 100 cc. of blood. The white blood cell count was within normal limits in all but two patients, who had a slight leucocytosis. The differential count was never unusual. The sedimentation rate, when done, was markedly elevated. The blood total cholesterol concentration was normal the few (three) times it was done. X-ray examination

Fig. 3 (Case 8).—Anisotropic crystal in a multinucleated giant cell. A small granuloma with several giant cells in lower portion of field. Polarized light with partial extinction; reduced ⅓ from mag. × 620.



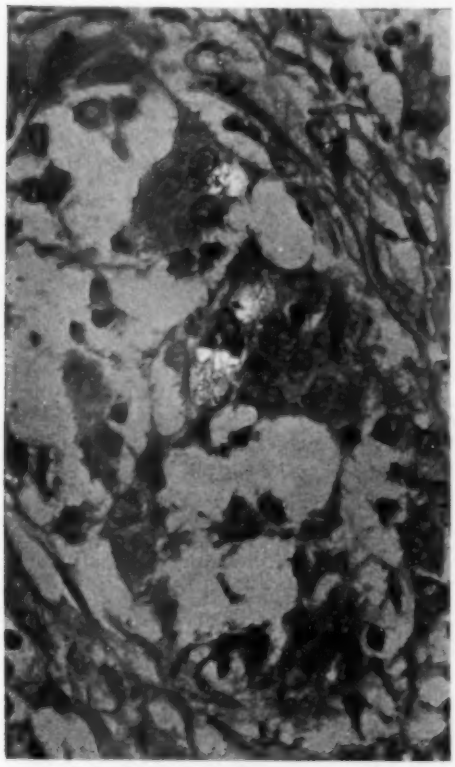


Fig. 4 (Case 4).—Many anisotropic crystals in multinucleated giant cells in a small granuloma. Polarized light with partial extinction; reduced ⅓ from mag. × 620.

of the chest showed marked compression of the trachea in one patient, who complained of marked pain, tenderness, dysphagia, and dyspnea.

A euthyroid state, as determined by clinical evaluation, was present in 10 cases. In three there was mild hyperthyroidism, while in a fourth patient an elevated basal metabolic rate was associated with exophthalmos.

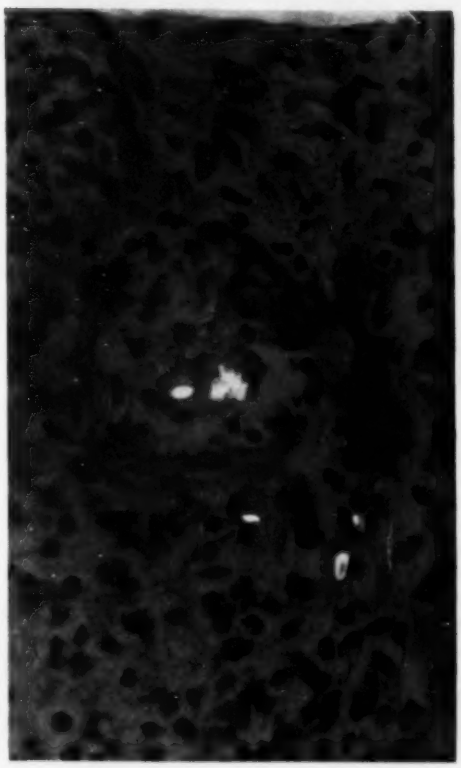
At operation adherence to the trachea and to the surrounding structures was found in eight cases. In some, excision was effected only by sharp dissection. Excisions varied from almost total thyroidectomy (performed in two cases) to partial resection of the involved lobe. All patients made an uneventful recovery postoperatively.

There were no recurrences in four patients one year postoperatively. Two of the group had mild hypothyroidism which improved with small doses of thyroid.

PATHOLOGIC DESCRIPTION

The thyroid glands, grossly, were firm to hard in consistency and cut with increased resistance. Histologically all cases were characterized by the presence of fibrosis and a granulomatous reaction associated with tubercle-like cellular aggregates. The histologic details found in the sections when examined by diffused light correspond with those described by de Quervain [3] and the other authors cited. However, with polarized light a striking observation was noted. Anisotropic crystalline material was prominently scat-

Fig. 5 (Case 3).—Anisotropic crystals in a partially disrupted acinus which shows eccentric cellular heaping. Some anisotropic material is seen in the interstitial tissues nearby. Polarized light with partial extinction; reduced ⅓ from mag. × 620.



tered in most sections (Figs. 2 to 6 ‡). These crystals appear identical with those studied and described by Richter § in normal thyroid glands. In the cases under discussion crystals were found in various locations. At times they were found embedded in intra-acinar colloid as in normal glands. At other times they were found within granulomata, either central or peripheral in location. A few were seen scattered in the dense reactive stroma (Fig. 5). An occasional crystal was found within a giant cell. Often the giant cells containing the crystals were part of a tubercle (Fig. 4), but at other times they were isolated in the stroma (Fig. 2).

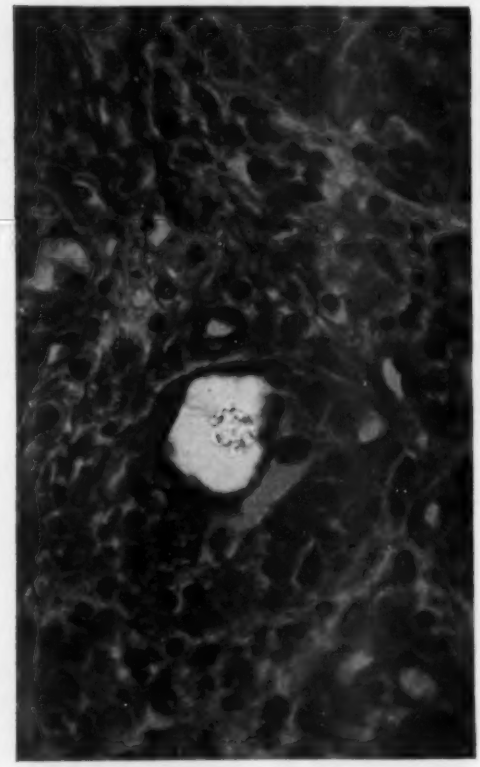


Fig. 6 (Case 7).—Large anisotropic crystal in the wall of an acinus and partially surrounded by epithelial and other mononuclear cells. Polarized light with partial extinction; reduced ⅓ from × 620.

The crystals found in these sections showed the same variation in size and pattern as those described by Richter.‖ The large plaque-like crystals were often fractured, and occasionally dislodged, presumably a result of sectioning.

COMMENT

The coexistence of crystals and granulomata, as well as their relative positions in the sections, suggest a causal relationship. The intra-acinar location is a "normal" one for crystals and here they incite no unusual reaction. However, many acini appear disrupted and the epithelium discontinuous. The basement membranes of acini in chronic thyroiditis are severely degenerated even in early stages. This finding, described recently by Sommers and Meissner,[10] was observed only in chronic thyroiditis and leads the authors to advance the thought "that chronic thyroiditis may develop as a sequel to degeneration of the basement membrane and local dissolution." Therefore, it may be further presumed that in granulomatous thyroiditis colloid and associated crystals escape into the thyroid interstitium and set up a granulomatous reaction.

It is well known that mineral crystalline anisotropic material, e. g., talc, as well as organic anisotropic material, e. g., suture material, or cotton may excite a granulomatous response often associated with multinucleated foreign-body giant cells.

Unpublished work has been done to show that calcium oxalate monohydrate is capable of inciting a granulomatous reaction associated with foreign-body giant cells. Crystals of this material inoculated into the muscles of the rat's thigh have given rise to typical granulomatous lesions.

Colloid itself has been considered a foreign body that may incite a giant-cell reaction.[6] A

‡ The photographs in Figures 2 through 6 have been taken under polarized light using an Opton polarizing filter, an Opton analyzing filter, and a compensating disc (Zeiss-Winkler). The photographs were taken with partial extinction in an attempt to show the background detail in addition to the anisotropic material.

§ References 1 and 2.

‖ References 1 and 2.

rounded central mass of colloid is often very prominent in the tubercles, and frequently this globule is closely circumscribed by giant cells and large mononuclear cells resembling epithelioid cells. De Quervain and Giordanengo [6] discussed this possibility and thought that "altered" colloid probably brought about the granulomatous reaction. A similar foreign-body reaction to material formed in acini may be seen elsewhere in the body, for example, in the prostate, where corpora amylacea are occasionally found surrounded by multinucleated foreign-body giant cells. On the other hand, Goetsch [11] expresses some doubt of the importance of colloid as an irritative factor in the causation of a giant-cell reaction.

It is possible, then, that both the "altered" colloid of the central mass as well as the calcium oxalate crystals may act as incitants of a granulomatous reaction.

Whether these giant cells are of epithelial or mesenchymal origin is not germane to the present discussion and no additional evidence is presented to elucidate this problem. However, some of these cells appear to be made up of coalesced epithelial cells, while others resemble foreign-body giant cells more closely. On occasion, each type has been seen bearing engulfed crystals. Now and then the bulk of the cytoplasm of such giant cells is occupied by one or several large crystals (Figs. 2, 6).

This discussion, it must be emphasized, concerns itself principally with certain histologic features of this lesion. The basic etiology is unknown. No specific organism has been isolated, and a virus has been implicated only on indirect evidence. In this series, two patients gave a definite history of infection of the upper respiratory tract immediately preceding the onset of the thyroid inflammation.

Excluding the foregoing 14 cases, the other 29 of the 43 cases reviewed are in sharp contrast, in that there is a significant absence of crystals. In 25 of 29 cases of nongranulomatous thyroiditis reviewed, representing a mixed group including "chronic thyroiditis," struma lymphomatosa, struma fibrosa, the hyperplasia of the thyroid with involution, crystals were absent in the slides studied. In the remaining four cases crystals were only occasionally seen. Three cases, in addition to features typical of Hashimoto's struma, possessed many multinucleated giant cells as well as a few structures resembling granulomata. In one of these cases typical crystals were seen in a giant cell. In the fourth case there were scattered areas of lymphocytic infiltration. In this case a few crystals were seen in the relatively unaltered acini but none were found in the lymphocyte-rich areas. In general, focal areas of involutional change seen in some thyroid glands are often devoid of crystals, while neighboring relatively unaltered acini may have scattered crystals embedded in the intra-acinar colloid.

SUMMARY AND CONCLUSIONS

The clinical features of 14 cases of granulomatous thyroiditis are summarized.

Anisotropic crystalline (calcium oxalate monohydrate) material was found in each of these glands, both within and outside of acini.

The crystals probably bear a relationship to the granulomatous reaction and the presence of multinucleated giant cells.

Twenty-five of 29 nongranulomatous cases variously diagnosed as "chronic thyroiditis," "Hashimoto's struma," "Riedel's struma," "lymphadenoid goiter," and "thyroid gland with hyperplasia and involution" did not contain any crystals. In the four remaining cases a few crystals were seen. In three there were features typical of struma lymphomatosa, as well as multinucleated giant cells and a few granulomata. In the fourth case the diagnosis was "hyperplasia of thyroid gland with involution."

The following physicians supplied clinical and pathologic material of some of these cases: Dr. J. W. Ehrlich, Dr. M. J. Fein, Dr. H. D. Kesten, Dr. T. Morrione, Dr. E. G. Ramsdell, and Dr. J. G. Sharnoff.

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News and Comment

ANNOUNCEMENTS

Annual Schering Award Competition.—The 10th annual Schering Award competition for medical students has opened, and titles of the following three subjects have been selected for which papers may be submitted: Current Concepts in the Management of Osteoporosis; Prevention and Treatment of Blood Transfusion Reactions, and Recent Trends in the Clinical Use of Adrenocortical Steroids. A first prize of $500 and a second of $250 will be awarded for each of the three subjects. Deadline for entry forms specifying the choice of title is July 1. Manuscripts should be mailed not later than Oct. 1. Students may compete individually or cooperatively in teams. Information and instructions for the award competition are available from the Schering Award Committee, 60 Orange St., Bloomfield, N. J.

Atherosclerosis

III. Fractional Distribution of the Esterified Fatty Acids of the Blood Lipids of Rabbits with Cream and Cholesterol Diets

EDWIN F. HIRSCH, M.D.
and
RICHARD NAILOR, Ph.D., Chicago

Rabbits, rats, guinea pigs, dogs, and chickens have been used in experiments to determine the factors responsible for the evolution of atherosclerosis and how this vascular disorder can be prevented or modified. Fed a diet to which cholesterol is added, these animals become hyperlipemic and lipids with a high content of cholesterol are deposited in the lining of the aorta, the arteries, and other tissues of the body. Because atherosclerotic lesions of the aorta and its branches can be produced experimentally in animals by feeding cholesterol, etiologic significance has been ascribed to dietary foods containing cholesterol in the evolution of human atherosclerosis. Objectors to this broad implication remind the experimenters that rabbits, the animals commonly used in the feeding experiments, absorb cholesterol from the intestinal tract in amounts far greater than the tissues are able to metabolize. The objectors emphasize that the hyperlipemia developing in animals fed thus is an unstable emulsion, and the lipids separating from the blood plasma are engulfed by phagocytes in the lining of the blood vessels and in other tissues where depositions occur. This deposition of the emulsified lipids seems to be related to the high content of cholesterol in the lipid complex of the dispersed particles.

Weinhouse and Hirsch [1] reported analyses of the lipids of the blood in rabbits that had become hyperlipemic with a diet containing cholesterol. In these analyses the total lipids of the serum were increased 11 times over the normal, and of the lipid fractions the free cholesterol was increased 25-fold; the cholesterol esters, 30-fold; the phospholipids, 7-fold, and the neutral fats, 5-fold. Clearly, in this disproportionate increase of the lipid fractions, the free and ester cholesterol fractions greatly exceeded the elevation of the phospholipids and of the neutral fat.

The neutral fats liquid at body temperature, such as triolein and others, are solvents for the lipids solid at body temperature, such as cholesterol, the cholesterol esters of stearic and palmitic acids, the phospholipids containing similar fatty acid radicals, and the triglycerides composed predominantly of these fatty acid groups. The lipids of the blood are transported in the plasma as emulsified particles of mixed chemical composition. For practical purposes they consist of three main classes: (1) the neutral fats, namely, the triglycerides of the saturated and unsaturated fatty acids; (2) the sterols (cholesterol) and their derivatives, and (3) the phosphatides, such as the cerebrosides and the ceramides.

All natural fats are mixed triglycerides, but each triglyceride usually is constituted chemically according to the simple principle proposed by Hilditch [2] and is referred to as the even, or the widest, distribution of the

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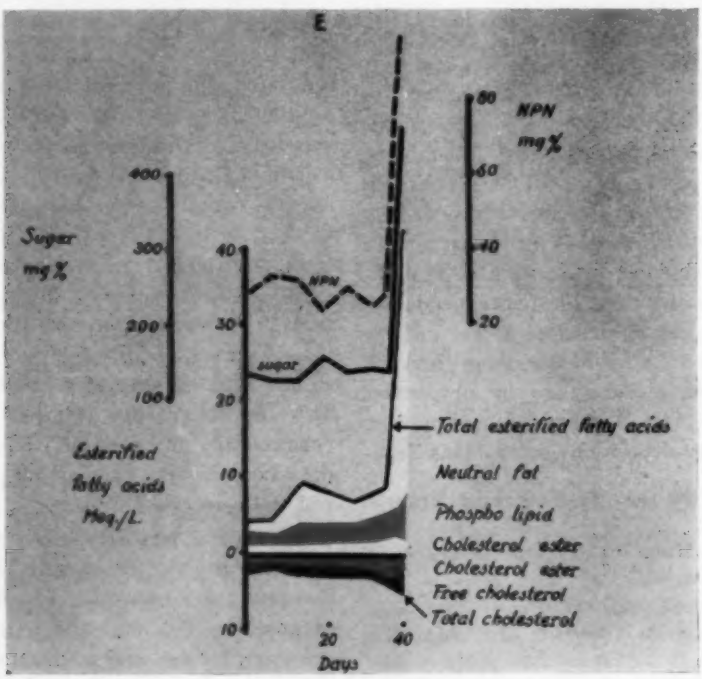
The Seymour Coman Fellow of the Department of Pathology of the University of Chicago (Dr. Nailor).

fatty acid groups between the glycerin molecules. According to this principle, as soon as a given fatty acid forms about 35% of the total fatty acids in a fat, it will occur at least once in every triglyceride molecule, and no simple triglycerides containing three groups of the same fatty acid will occur until such acid forms nearly two-thirds or more of the total fatty acids. Each fatty acid acts independently of the others in competing for union with glycerin according to this general plan. The formula fails to describe accurately the general triglyceride structure of animal fats in only two classes: (1) animal depot fats unusually rich in mixed glycerides containing stearic groups and (2) milk fats in which the lower saturated acids from butyric to lauric are in appreciable amounts (13%). The neutral fats, accordingly, are triglycerides of mixed fatty acid composition, each molecule containing three fatty acid radicals.

The significant sterol of the blood, cholesterol, is chemically a monatomic alcohol, solid at body temperature. It combines with a single fatty acid to form an ester compound. The cholesterol esters containing the higher saturated fatty acids, such as stearic, are solid at body temperature; those of the lower unsaturated fatty acids (olein) are liquid or semiliquid. Cholesterol esters normally form 70% to 75% of the total serum cholesterol. The fatty acids in these cholesterol esters are derived, probably, from the neutral fats, and each cholesterol ester molecule contains one fatty acid group.

The monoamino phosphatides, lecithin and cephalin, are choline and cholamine phosphoric acid esters which have replaced respectively one of the three fatty acid radicals of a triglyceride fat. These diglycerol esters, lecithin and cephalin, thus are related chemically to neutral fat. According to Thann-

Fig. 1.—Graphs of the analyses of the lipids, the sugar, and the nonprotein nitrogen of the blood of a rabbit given supplemental feedings of 36% cream for 40 days. All lipid fractions increased in amounts but, as the total increased, and especially in the acute terminal phase, the neutral fats had the largest increase. Graphs also illustrate the abrupt hyperglycemia and azotemia in the acute terminal illness.



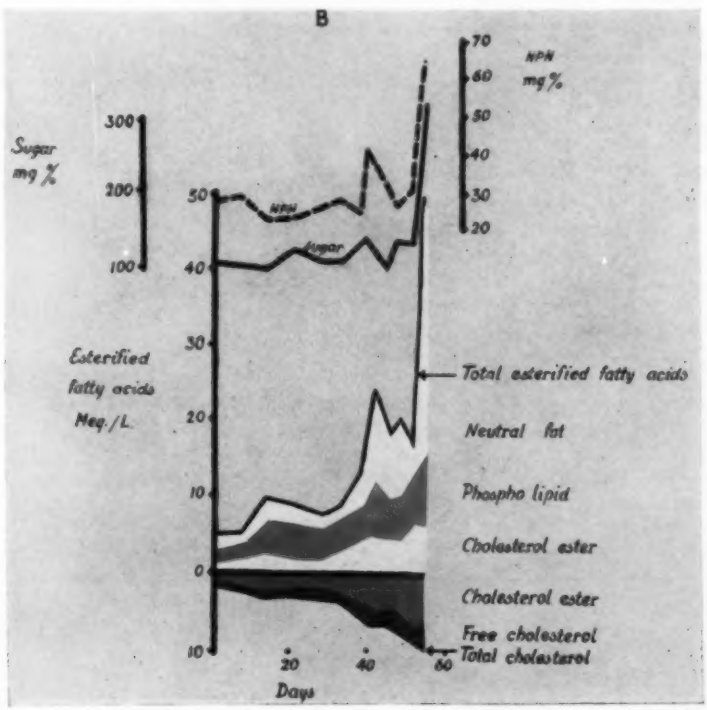
hauser,[3] 70% to 90% of the total phospholipids of the serum may be evaluated as lecithin.

These comments emphasize that the esterified fatty acids form a considerable and significant amount of the total lipids of the blood in all fractions except the free cholesterol. Methods for measuring colorimetrically the cholesterol content in small amounts of blood serum have been in use for many years. The availability as well as the simplicity and widely tested use of these methods for measuring the serum cholesterol contrast with the more elaborate methods required and the much larger amounts of serum needed for measuring the other lipid fractions of the blood. The general practice of estimating the cholesterol content of the serum in various diseases, the ease of analysis, and the emphasis placed on the cholesterol content of the serum as a cause of atherosclerosis have focused attention on the cholesterol content of serum in various diseases without realizing fully that the other lipid fractions could fluctuate dependently or independently. In 1949 Bauer and Hirsch [4] published a method for the colorimetric determination of the total esterified fatty acids in small amounts of serum, and more recently this method has been reevaluated, modified slightly, and more closely standardized.[5] The method has been used in studies of the blood lipids in normal human adults,[6] in infants and children,[7] in patients with diabetes,* in children with fibrocystic disease of the pancreas,[7] and in rabbits with experimentally produced disorders.† These studies disclose that analyses establishing the distribution of the esterified fatty acids in the three main classes of blood lipids demonstrate wide

* References 8 and 9.

† References 10 through 12.

Fig. 2.—Graphs of the analyses of the lipids, the sugar, and the nonprotein nitrogen of the blood of a rabbit fed 36% cream supplementally for 55 days. In the abrupt elevations of the total esterified fatty acids the neutral fat fraction had the largest increase.

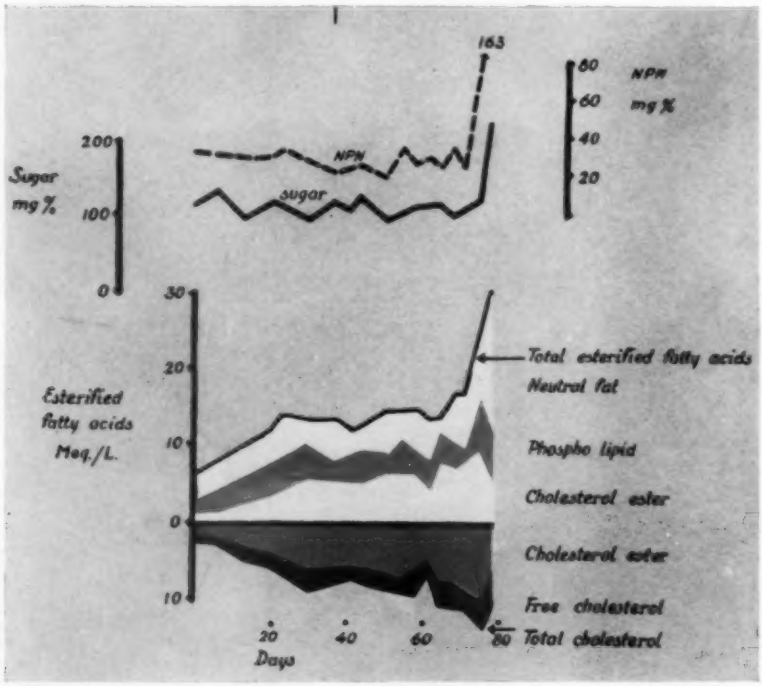


variations from the normal fasting level in the total amounts of the serum lipids and in the aliquots of the total.

The composition of the emulsified lipids in the blood at the fasting level and in the various forms of hyperlipemia can be likened to a solvent and its solutes in varying proportions. At the one extreme, the solvent is in excess, as with the simple postprandial hyperlipemia, the early phases of idiopathic hyperlipemia, most forms of diabetic hyperlipemia, and some of the lipemias associated with nephritis in the human (nephroses) or produced experimentally in animals. At the other extreme, the solutes approach the supersaturation level, as in the experimental feeding of cholesterol to rabbits and other animals with the production of atherosclerosis, and in diabetics or other patients with xanthomatosis. Persistence of the imbalance when the lipid solute is in excess, as with the cholesterol diets in rabbits, results in the deposition of lipids containing large amounts of cholesterol in the lining of the aorta and in many other tissues of the body. This deposition is associated with a wasting nutritional disorder and cachexia of the animals.

The effects of feeding lipids containing a large content of the solvent liquid fats on the lipids of the blood and other tissues in rabbits, the test animal for so many studies on the feeding of cholesterol, have not been investigated. Cook and Thomson,[13] from observations on the absorption of fat and cholesterol in the rat, guinea pig, and rabbit, concluded that at levels up to 20% of the diet all fats are readily absorbed and that absorption is better with fats containing the glycerides of the unsaturated fatty acids. At a dietary level of 16% fat, the guinea pig is less efficient than are the rabbit and the rat, and the absorption of olive oil in the latter two animals

Fig. 3.—Graphs of the analyses of the lipids, the sugar, and the nonprotein nitrogen of the blood of a rabbit with supplemental feedings of 36% cream for 78 days. All fractions of the lipids participated in this, the neutral fat increasing abruptly in the terminal phase along with the acute hyperglycemia and azotemia.



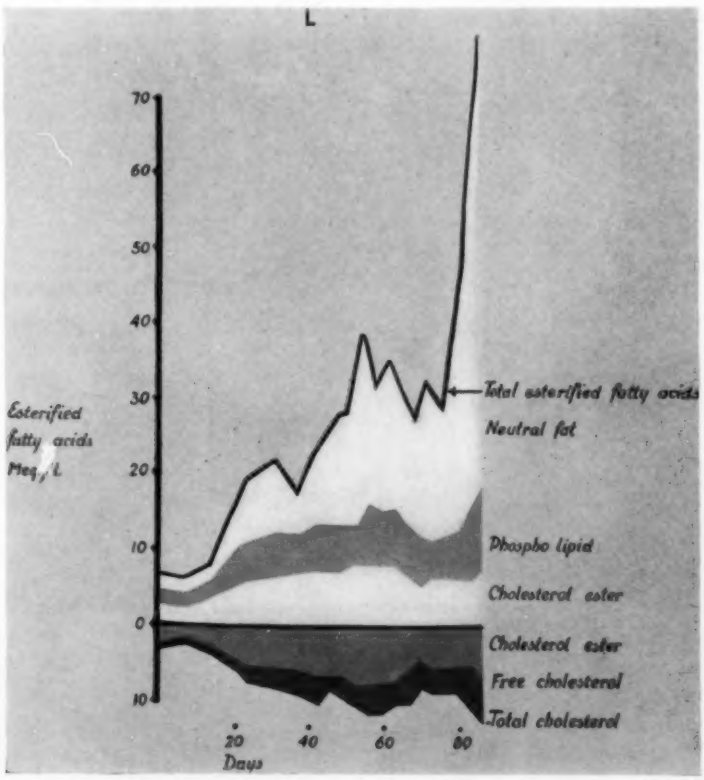
is of the same order as occurs in man. Cook and Thomson stated that the rabbit is unlikely to encounter cholesterol in its normal food, but it seems to possess a mechanism well adapted for the absorption of this substance. With a standard diet containing 16.6% olive oil, they reported that rabbits absorb about 94% of intake fat, rats 92%, and guinea pigs 77%. According to Hilditch,[2] a fat usually can be assimilated if it is almost completely fluid at the body temperature of the animal (40 C).

Experiments in which rabbits received a regular diet supplemented by quantities of emulsified fat (cream), with a high content of the solvent group (olein) rather than of the solute group (cholesterol), were made. The cream used contained about 36% fat of mixed composition as given in the following tabulation:

	Per Cent
Butyric acid	2.9
Caproic acid	1.9
Caprylic acid	0.8
Capric acid	1.6
Lauric acid	5.8
Myristic acid	19.8
Palmitic acid	15.2
Stearic acid	15.0
Oleic acid	31.9
	94.9

The cream contained 0.1% total cholesterol and 0.3% to 0.4% phospholipids. It was given to large rabbits by gavage daily, Saturday and Sunday excepted, in amounts of 15 to 50 cc. The rabbits otherwise had the usual balanced diet without restriction in amount.

Fig. 4.—Graphs illustrating the distribution of the fractions of the blood lipids in a rabbit with supplemental feeding of 36% cream for 85 days and where the lipemia was maintained at a high level for more than two months. Note that at these levels all fractions participated in the increase, but that of the neutral fat fraction was the largest.

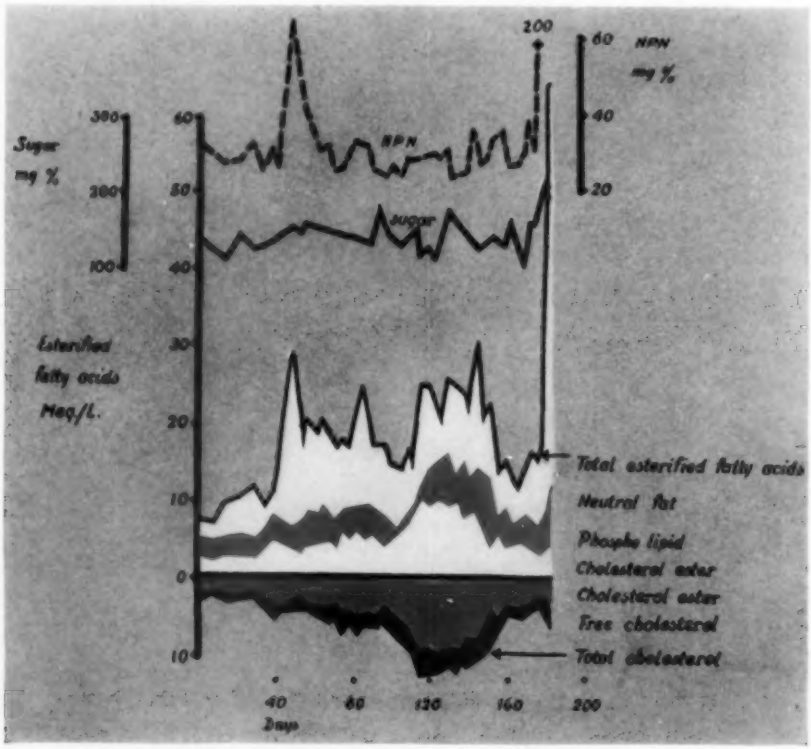


The total esterified fatty acids of the blood were determined according to the method of Nailor, Bauer, and Hirsch,[5] the total and esterified cholesterol according to Schoenheimer and Sperry.[14] After the lipid extract had been digested with sulfuric acid and hydrogen peroxide, the lipid phosphorus was determined by the method of Fiske and Subbarow [15] adapted to spectrophotometric analysis. Milliequivalents of fatty acid combined with lipid phosphorus were calculated according to the formula described by Peters and Man [16]: Phospholipid fatty acid (mEq/liter) = lipid phosphorus (mEq/liter) × 1.8. Neutral fat was calculated as follows: Neutral fat = total esterified fatty acid—(cholesterol ester fatty acid + phospholipid fatty acid). All values were expressed as milliequivalents per liter (mEq/liter).

These analyses of the serum lipids provided the data for establishing the distribution of the esterified fatty acids in the neutral fat, the phospholipid, and the cholesterol ester fractions. The fasting level of the esterified fatty acids of the blood in rabbits ranges between 4 to 8 mEq/liter. The sugar and the total nonprotein nitrogen content of the serums used for lipid analysis also were determined.

With cream given daily in the amounts mentioned there is, after about 7 to 10 days, an elevation of the total esterified fatty acids of the blood. If the quantity of cream fed daily is in amounts so that the increase of the esterified fatty acids of the blood does not progress rapidly beyond a critical level of 20 to 30 mEq/liter, the rabbit seems to maintain his normal state of health. When the feedings

Fig. 5.—Graphs of the lipids, the sugar, and the nonprotein nitrogen of the blood of a rabbit given 36% cream for 180 days. All lipid fractions were increased, but at the higher levels the increase of the neutral fat fraction was the largest and, terminally, far more than the others.



cause a further elevation of the esterified fatty acids of the blood lipids, the rabbit becomes listless, develops diarrhea, and, if the feedings are continued, the esterified fatty acids rise suddenly to a much higher level; and in this final acutely critical state the nonprotein nitrogen and the sugar of the blood rise along with the increase of the esterified fatty acids and the animal succumbs.

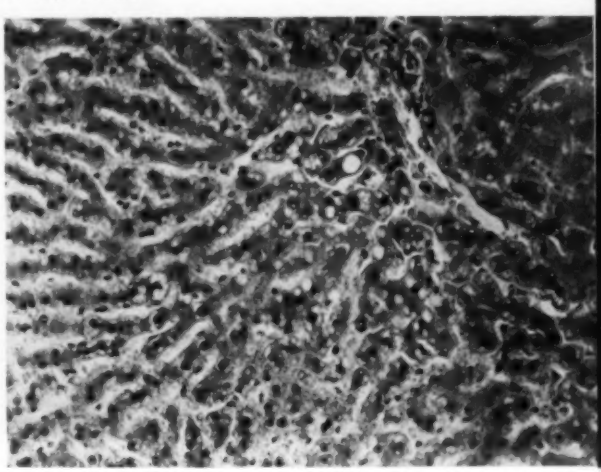
Figures 1 to 5 graphically present the results of the blood serum analyses in five rabbits with supplemental feedings of cream for 40, 55, 78, 85, and 180 days. The graphs in these Figures show the gradual increase in the amount of the esterified fatty acids of the blood; their distribution in the various lipid fractions; the gradual increase of each of the lipid fractions; the abrupt rise of the esterified fatty acids, chiefly those of the neutral fat fraction in the final critical phase, and the associated azotemia and hyperglycemia. With the slight and, perhaps, even moderate increase in the total esterified fatty acids of the blood, all three fractions of the lipids participate, but at higher levels the neutral fat fraction is increased much more than the others. When there is an abrupt rise in the acute terminal stage with the marked increase of the total esterified fatty acids, the neutral fats, especially, are markedly elevated; the other fractions also are increased, but much less in amount.

Examinations of the tissues of animals killed or dying in the acute terminal phase after 40 to 180 days of feeding disclosed no deposits of lipids in the lining of the aorta, large arteries, or other important viscera, except the liver. The liver cells in histological preparations were markedly vacuolated (Fig. 6), and sections of formalin-fixed tissues stained with Sudan had marked deposits of lipids in the hepatic cells. The tubular epithelium of the kidneys focally had fatty changes and the myocardium had similar focal fatty changes in the muscle fibers. The lining of the aorta and of the large arteries had no fatty deposits, the spleen and lungs contained only a few large mononuclear phagocytes with small droplets of lipid material, and the reticuloendothelial system throughout was not laden with lipid deposits.

COMMENT

The rabbit absorbs cream fat from the intestinal tract when it is given daily by gavage as a supplement to a regular balanced diet. After a latent period of about a week with an intake of 15 to 50 cc. of cream daily, the lipids of the blood are elevated. Then follows a gradual progressive increase of the serum lipids, as the supplemental feeding of the cream continues, determined by the amounts given. If the supplemental feeding of cream is omitted, the hyperlipemia disappears. With persistence of the supplemental feeding of cream and if increased, the total esterified fatty acid content of the serum may increase four- to six-fold. All fractions of the serum lipids, that is, the neutral fats, the phospholipids, and the free and the esterified cholesterol, participate in this gradual elevation. This is then a persistent form of hyperlipemia, in contrast with the transient postprandial hyperlipemias. The increase of the cholesterol and phospholipids in the complex of serum lipids probably is from endogenous sources. Approximately the normal ratio of free to esterified cholesterol is maintained in the elevated total cholesterol fraction. When the amount of total serum lipids ranges into the higher levels in these feeding experiments, the neutral fat fraction is increased more than the others. As the feeding experiment continues and the total esterified fatty acid

Fig. 6.—Photomicrograph illustrating the fatty changes of the liver cells in the terminal phase of rabbits made hyperlipemic by supplemental feeding of 36% cream.



content of the serum ranges above a critical level of approximately 20 to 30 mEq/liter, the rabbit becomes acutely ill with diarrhea; prostration; a marked and abrupt rise of the blood lipids, especially of the neutral fat fraction; an azotemia, and a hyperglycemia. In this stage the animal dies. This acute metabolic disturbance produced in rabbits by the supplemental feeding of cream is unlike the chronic cachexia or wasting disorder associated with the hyperlipemia which occurs when cholesterol is added to the food of rabbits.

The chronic hyperlipemia produced in rabbits by the supplemental feeding of 36% cream, in the amounts given, is not associated with lipid deposits in the lining of the aorta, the arteries, and the reticuloendothelial system of the animal. This contrasts with the changes described in the tissues of rabbits fed cholesterol. Cream is mainly a triglyceride solvent; cholesterol and its esters are solutes in the blood lipid complex. The serum lipids, with the hyperlipemia of the rabbits fed cream, have a high content of solvent neutral fat, and the lipid particles of this composition do not stimulate a hyperplasia of the reticuloendothelial phagocytes or form deposits of lipophages in the lining of blood vessels (atheromas) and in other tissues of the animal. The composition of the serum lipids in the hyperlipemia of rabbits fed cholesterol differs. According to the analyses, the total lipids of the serum of these rabbits may increase as much as 11 times over the normal. However, the solvent neutral fat fraction of the lipids is increased only 5-fold; the phospholipids, 7-fold, and the cholesterol fractions, 25- to 30-fold (Fig. 7). The lipid material of this composition stimulates profoundly the reticuloendothelial system of the body, and large lipophages laden with cholesterol and/or its esters are deposited as atheromas in the lining of the aorta, the arteries, and in many other tissues of the body. The difference in the effects of these chemically dissimilar hyperlipemias on the reticuloendothelial and other tissues of the rabbits is marked. Several conclusions are suggested. Serum lipids rich in a solvent fat, such as triolein, can be metabolized in the rabbit without leaving appreciable residues for phagocytosis and tissue

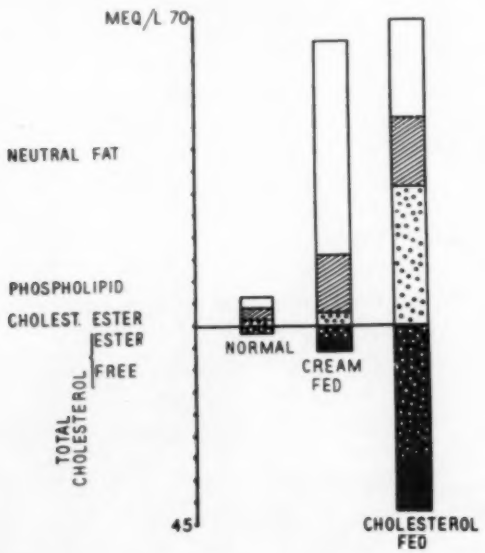


Fig. 7.—Graphs illustrating the distribution of the fractions of the lipids in the serum of a rabbit on a balanced diet without cream compared with the lipid fractions in the hyperlipemia of rabbits fed cream and of rabbits fed cholesterol.

deposition. Conversely, serum lipids with a disproportionately large amount of solute, such as cholesterol, lead to lipid deposits in the aorta and other tissues of the animal which, like a particulate foreign body substance, stimulate mononuclear cell phagocytosis and, in loci of deposition, even may cause proliferation of fibroplastic or scar tissues.

The aorta of a rabbit made hyperlipemic by supplemental feedings of solvent fat does not become atherosclerotic. However, the aorta and the blood vessels of a rabbit, made hyperlipemic by adding cholesterol to his diet, become atherosclerotic and his reticuloendothelial tissues respond, as with a particulate foreign body circulating in his blood stream. Analyses disclose that the serum lipids of this rabbit have a small content of solvent fat and a disproportionately large amount of the solute substance (cholesterol). Perhaps blood lipid systems, originally with the various fractions in proper proportions, after deposition may become disproportionate by utilization of the solvent lipid faster than the solute lipids.

The pattern of lipid distribution of the hyperlipemia in the final acute phase with death in rabbits fed cream and associated with azotemia and hyperglycemia is similar to the distribution of the lipids in the hyperlipemia of a diabetic patient in coma.[17]

SUMMARY

Rabbits given 15 to 50 cc. of 36% cream daily by gavage as supplement to a regular diet become hyperlipemic in about a week or 10 days.

The neutral fat, the phospholipids, and the total cholesterol fractions participate in the lipid increase during this stage of the hyperlipemia.

As the daily feeding of cream continues and, especially, with larger amounts, the hyperlipemia increases and at these higher levels the neutral fat fraction increases more than the others.

When a level of about 20 to 30 mEq/liter total esterified fatty acids in the blood is exceeded appreciably, the rabbit has an acute metabolic disturbance with diarrhea, a sudden marked rise of the esterified fatty acids of the blood, chiefly of the neutral fat fraction, a hyperglycemia, an azotemia, and the animal dies.

This acute metabolic disorder with a hyperlipemia in which the lipids of the blood have a large neutral fat fraction contrasts sharply with the chronic wasting disorder observed in rabbits fed cholesterol whose hyperlipemic blood has a 25- to 30-fold increase of cholesterol or its esters, a 7-fold increase of the phospholipids, and only a 5-fold increase of the neutral fat fractions.

The physical composition of the lipids of the blood can be likened to a solvent and its solutes. The solvents of the blood lipids are chiefly the neutral fats (olein); the solutes are cholesterol, its esters, and other lipids solid at body temperature.

The blood lipids in the hyperlipemia produced in rabbits with a cream diet have a large content of neutral fat (solvent). Lipids of the blood with this composition are not deposited in reticuloendothelial tissues and blood vessels. The composition of the serum lipids in the hyperlipemia of rabbits fed cholesterol differs. The total lipids of the serum of these rabbits may increase as much as 11 times over the normal. However, the solvent neutral fat fraction is increased only 5-fold, the phospholipids 7-fold, and the cholesterol fractions 25- to 30-fold. The lipid material of this composition stimulates the reticuloendothelial tissues of the body, and large lipophages laden with the lipids are deposited as atheromas in the lining of the aorta, the arteries, and many other tissues of the body. The differences in the effects of these chemically dissimilar hyperlipemias on the reticuloendothelial and other tissues of the rabbit are marked.

Blood lipid systems originally with the various fractions in proper proportions, after deposition in tissues, may become disproportionate by utilization of the solvent lipid faster than the solute lipids or, by solvent action, accumulate an excess of the solutes.

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Responses of the Liver to Injury

Effects of Acute Carbon Tetrachloride Poisoning

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The responses of the liver to injury and the reparative phenomena which follow present a basic and challenging problem. Earlier studies have described the histological sequelae to hepatic injury induced by a hepatotoxin.* Within recent years interest has been stimulated in the nucleic acids as indices of tissue growth and regeneration. The development of cytochemical † and chemical ‡ methods for the analysis of these compounds has made it possible to study simultaneously the alterations in tissue nucleic acids by several techniques.§ Accordingly, the serial changes of the rat liver in response to an acute injury have been studied by histological, spectrophotometric cytochemical, and chemical methods. The results indicate a characteristic pattern of response to acute injury and permit the subsequent study of the effects of agents designed to modify this pattern. In addition, a critical comparison of the usefulness of each of these three methods of analysis is presented.

From the Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, and the Department of Zoology, Columbia University.

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* References 1 and 2.

† References 3 through 5.

‡ References 6 through 9.

§ References 10 and 11.

METHODS

Male rats, about 200 gm. in weight, were obtained from the Wistar Institute. They were caged individually and were fed Purina Checkers ad libitum. Carbon tetrachloride was diluted with liquid petrolatum and a single dose of 0.066 cc. of CCl_4 per 100 gm. of body weight was injected intraperitoneally. The animals were killed after 24, 48, 72, 96, and 120 hours, by a blow on the head. The liver was removed rapidly, sliced, blotted free of blood, and weighed. Samples were fixed in Carnoy's solution and in 10% formalin for histological and spectrophotometric cytochemical studies. Other samples were weighed and then homogenized and prepared for chemical analysis in a cold room (4 C). The tissues were in the cold homogenizers within two to three minutes after removal from the animal.

Histological Techniques.—Carnoy-fixed tissue was embedded in paraffin and sections (5μ-10μ in thickness) were stained with the Giemsa dye and by the Hotchkiss periodic acid method.[12] The Giemsa-stained sections were studied for the general cytoarchitecture of the organ and for cellular basophilia. The loss of cytoplasmic basophilic clumps and the development of intense cytoplasmic eosinophilia in necrotic cells was strikingly demonstrated by this stain. The periodic acid method was used for the demonstration of glycogen. Formalin-fixed specimens were sectioned frozen and were stained for fat with oil red O.

Spectrophotometric Cytochemical Techniques.—The amounts of deoxyribonucleic acid (DNA) per nucleus were determined by measuring photometrically the light absorption by Feulgen-stained nuclei. The light source was a Mercury AH 4 lamp, 564 mμ band, isolated with a Wratten 62 filter. The Carnoy-fixed tissues were cut at 15μ to 20μ thickness, and hydrolysis was conducted in the presence of 1N HCl at 60 C for 13 minutes. Sections were stained for one hour in reagent made up according to Stowell.[13] The apparatus was similar to that described by Pollister and Ris[4] and Swift.[5] The measurements were made through a central core

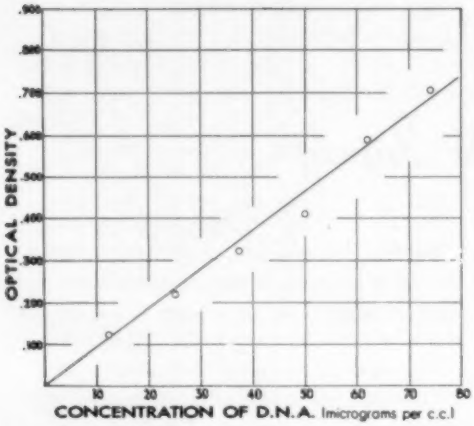

Fig. 1.—Relationship of optical density to concentration of DNA is shown.

of whole nucleus. The radius of the core was approximately 60% of that of the nucleus. The amounts of DNA, expressed in arbitrary units, were calculated according to Swift.[5]

Chemical Techniques.—Three to six grams of fresh liver was homogenized in the cold (4 C) in about 15 cc. of 5% trichloroacetic acid (TCA) and then extracted three times with about 30 cc. of 5% TCA to remove the acid-soluble fraction. At room temperature the lipid moiety was extracted from the residue by treatment with 30 cc. of an ether-95% alcohol (1 : 3) mixture three to eight times until the supernatant was colorless. The precipitate was washed with absolute alcohol and then with anhydrous ether. It was dried in a vacuum oven at 45 C to constant weight. In this dried state the powder could be kept indefinitely.

Samples of dried powder (20 to 30 mg.) were extracted three times with 3 cc. of 5% TCA at 90 C for 10 minutes. This volume of TCA, the number of extractions, and the time required for each extraction were checked experimentally and were found to give optimal yields of nucleic acids. The total extract was brought to 10 cc. with TCA, and aliquots were analyzed for nucleic acids. If kept stoppered in the cold (4 C), the extracts were stable for two days for deoxyribonucleic acid analysis and for five days for ribonucleic acid analysis.

Deoxyribonucleic Acid (DNA).—The method employed was modified from one obtained from Dr. Samuel Graff, Professor of Biochemistry, Columbia University College of Physicians and Surgeons, and is similar to that published recently by Ceriotti.[9] Aliquots (0.20 cc.) of the hot TCA extract were added to 0.2 cc. of 5% CHl and 0.02 cc. of 2% indole in 95% alcohol.‖ The mixture tube was loosely stoppered and heated in a boiling water bath for 10 minutes. It was cooled in running tap water, shaken with 0.6 cc. of chloroform, and centrifuged for three minutes at 35 g. The top aqueous layer was transferred to a microcuvette and was read at 494 mμ in the Beckman spectrophotometer against a reagent blank. The color was stable for two hours. A curve for the conversion of O. D. readings to concentration of DNA (Fig. 1) was made from a purified DNA (7.9% P) sample derived from mouse tumor.¶

Duplicate powder samples from each animal were analyzed in triplicate. The variations in replicate analyses for a single powder specimen were less than 5%. Results are expressed as DNAP per unit of dry and wet weight of liver tissue.

Ribonucleic Acid (RNA).—The method employed was that of Dische [8] adapted to 0.2 cc. samples of unknown.# Originally, with each lot of analyses, standards of RNA (Schwarz Laboratories, Mt. Vernon, N. Y.) and D-ribose were run along with the glucose, TCA, and water blanks. Later the RNA standard was omitted and the D-ribose standard was used as the reference point. From a series of 132 experiments at 42 different concentra-

‖ Indole was kept in an amber bottle in the cold (4 C) and was prepared freshly biweekly.

¶ This material was supplied by Dr. Samuel Graff.

Several procedures were found to be helpful in applying this method. The reaction was performed in 10 by 70 mm. test tubes, which were kept in vertical positions by being set into 3 cm. deep holes in a wooden block. In this manner, the concentrated H_2SO_4 could be added from a Koch burette without splashing against the sides of the tube. The reaction mixture was permitted to cool in air for one hour and then the cysteine was added.

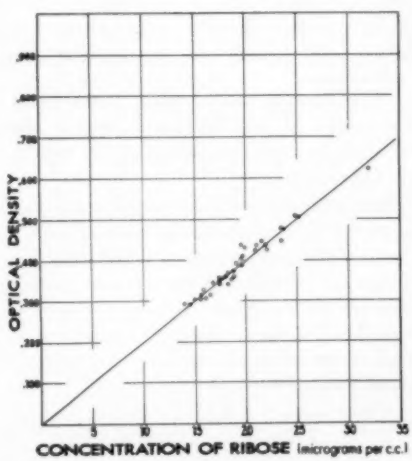

Fig. 2.—Relationship of optical density to concentration of ribose is shown.

TABLE 1.—*Summary of Clinical and Chemical Data*

Groups		Liver							DNAP				RNAP				Nitrogen	
Hours After CCl_4	No. of Rats	Δ Body Wt., Per Cent	Wt., Gm.	SEM	Per Cent Body Wt.	Per Cent Dry Wt.	SEM	Total Dry Wt., Gm.	γ/Mg., Dry	SEM	Mg./ 100 Gm., Wet	SEM	γ/Mg., Dry	SEM	Mg./ 100 Gm., Wet	SEM	Mg./ Mg., Dry	Gm./ 100 Gm., Wet
0	28	0	8.01	.14	4.00	21.1	.47	1.69	0.85	.01	18.30	.31	4.57	.10	95.92	1.94	.131	2.76
24	10	—6.5	9.43	.39	4.76	20.5	1.39	1.93	0.88	.02	17.99	1.58	4.80	.17	97.53	5.85	.129	2.65
48	23	—2.3	9.84	.22	4.34	17.4	.56	1.71	1.40	.03	23.76	.81	6.20	.11	106.96	3.32	.128	2.23
72	9	—4.0	10.40	.44	4.93	15.1	.83	1.57	1.15	.05	17.06	1.25	4.68	.16	84.62	3.58	.131	1.98
96	10	—3.5	9.07	.53	4.07	20.2	.38	1.83	0.92	.05	19.33	1.34	4.87	.21	96.66	3.94	.128	2.59
120	10	+1.0	9.51	.38	4.18	21.7	.50	2.06	0.92	.04	18.85	.42	4.59	.14	99.78	3.96	.124	2.69

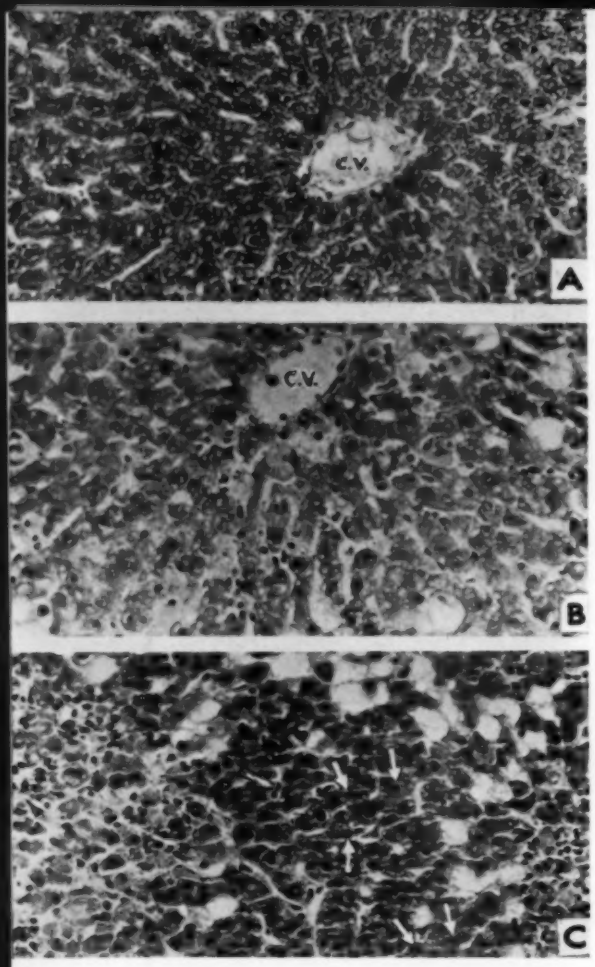

Fig. 3.—The following photomicrographs (Figs. 3 and 4) are representative of animals from each group. Giemsa stain; reduced about ¼ from mag. × 252. *C.V.* indicates central vein.

A, normal liver. Cytoplasmic basophilic clumps are prominent.

B, 24 hours after CCl_4. Necrotic cells and poorly stained surviving cells fill the pericentral zone.

C, 48 hours after CCl_4. Necrotic cells, intermingled with mononuclear elements, are to the left. The arrows indicate cells with mitotic figures.

tions of D-ribose, the curve in Fig. 2 was derived. While the D-ribose curve could not be used as a standard, such as the DNA curve (Fig. 1) was used, it served as a check on the validity of daily analyses. Thus, if the ribose standard for any day was off the curve (Fig. 2), the entire set of analyses was repeated. In a series of 26 experiments at six different concentrations, 1γ of D-ribose was found to be equivalent to 4.73γ of RNA. With this factor, the data were converted from D-ribose to RNA and, since the P content of this particular lot of RNA was found to be 10%, the results could be expressed as γ RNAP per unit of weight of liver tissue.

Dische[8] has discussed the details of this method and the reasons for using a known standard of D-ribose with each run of unkowns. Extreme care must be given each step in the analysis.

Duplicate powder samples from individual animals were analyzed in triplicate. These checked within less than 3%.

Nitrogen (N).—Aliquots of pooled liver powders from each group of rats were analyzed in duplicate by the semimicro Kjeldahl method. The total N values represent residual protein N plus nucleic acid N. Since the latter figure is only approximately 10% of the total N figure, the total N values may be considered to be largely protein N. The replicate nitrogen analyses checked within less than 2%.

RESULTS

Early in the period after CCl_4 injection, all animals lost weight, which they subsequently regained (Table 1). Since food intake was not measured, it is not known whether reduced intake was responsible for this weight loss. All animals remained active, sleek, and vigorous during the entire experimental period. Icterus was never observed.

Histological.—While the lesions produced by this hepatotoxin have been reported before,* they will be reviewed in order that the cytochemical and chemical data may be considered in relation to the concomitant structural changes. The livers of the control animals were normal in all respects and free from evidences of disease (Fig. 3*A*).

Twenty-Four Hours (Fig. 3*B*).—There was central necrosis involving one-fourth to one-half of each lobule. Cytoplasmic basophilia disappeared from the involved cells, often leaving an eosinophilic cytoplasm. Many cells were in a state of dissolution, with pyknotic nuclei. The affected areas contained a few polymorphonuclear and mononuclear cells. Peripheral to the zone of necrosis was a rim of greatly enlarged liver cells, filled with large cytoplasmic vacuoles. The periportal liver cells contained small vacuoles in their cytoplasm. No mitoses were seen. Glycogen appeared only in the periportal liver cells. Fat stains showed heavy droplet deposits in the cytoplasm of

* References 1 and 2.

the pericentral cells. The large vacuoles in the midzonal liver cells could not be stained with oil red O. The periportal liver cells showed occasional tiny fat droplets. This was the stage of necrosis without histological evidence of repair.

Forty-Eight Hours (Fig. 3*C*).—The pericentral zone of necrotic cells was heavily infiltrated by polymorphonuclear and mononuclear cells. Adjacent to this zone were large, vacuolated liver cells, similar to those seen at 24 hours (Fig. 3*B*). The surviving periportal liver cells were increased in size and their nuclei were swollen. Their cytoplasm had lost its basophilic granules and now contained small vacuoles. There were about five mitoses per high-power field in the periportal zone. Glycogen was stained in the periportal cells, except for those in mitosis. Fat was found in the necrotic liver cells as well as in the surviving periportal liver cells. The large vacuoles in the midzonal cells did not stain with oil red O. In this stage, the inflammatory reaction in the necrotic zone and the mitotic activity of the surviving cells were the most prominent features.

Seventy-Two Hours (Fig. 4*D*).—The central zone was filled with new liver cells and a few mononuclear cells. These hepatic cells were smaller than normal and contained a finely granular basophilic cytoplasm in contrast to the coarse basophilic clumps seen in normal liver cells. An occasional cell was in mitosis. A rare necrotic cell was seen in this pericentral zone. Sometimes the pericentral sinusoids were distended with blood and appeared as "lakes." The midzonal and periportal cells contained small vacuoles as well as coarse basophilic clumps in their cytoplasm. Glycogen was seen in all except the pericentral rim of cells. Fat droplets were seen in all the liver cells of the lobule. At this stage, the necrotic cells were replaced by new liver cells and a few inflammatory cells were present.

Ninety-Six Hours (Fig. 4*E*).—A few mononuclear cells persisted about the central vein. Some liver cells contained small clear vacuoles. Very rare mitotic figures were seen in the liver cells of the pericentral zone. The remaining hepatic cells contained coarse basophilic clumps. Glycogen was stained in all of the hepatic cells but only an occasional pericentral liver cell contained fat. Few remnants of injury were evident at this time.

One Hundred Twenty Hours (Fig. 4*F*).—Restitution to normal was complete except for very rare mononuclear cells and a few small liver cells with finely granular basophilic cytoplasm about the central vein. A very rare mitotic figure was still seen in this zone. Glycogen was present in all hepatic cells and fat droplets were observed only in occasional pericentral liver cells.

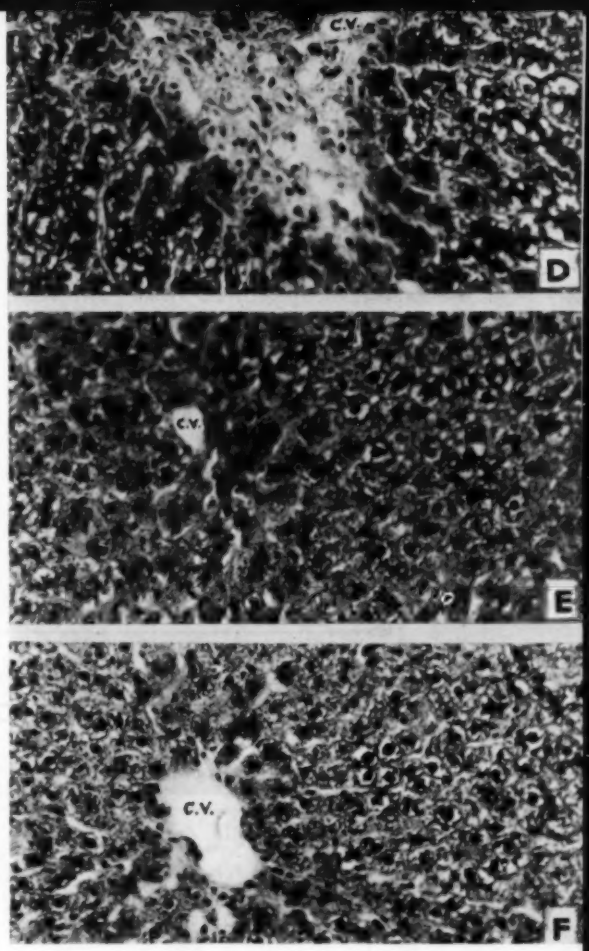

Fig. 4.—*D*, 72 hours after CCl_4. The pericentral zone has been cleared of necrotic tissue and contains liver cells and inflammatory tissue.

E, 96 hours after CCl_4. The tissue restoration is complete except for the presence of a few pericentral inflammatory cells.

F, 120 hours after CCl_4. The liver appears normal.

In summary, at 24 hours after the single dose of CCl_4 there was necrosis, at 48 hours the inflammatory and regenerative phenomena were most marked, at 72 hours restitution of the destroyed tissue had been largely accomplished, and by 120 hours only rare remnants of the previous damage remained. The accumulation of fat occurred first in damaged liver cells and later affected the surviving periportal cells. Some of the newly formed cells contained fat, too. Glycogen staining was lost by all injured (pericentral) cells and seemed to be reduced in the surviving liver cells. It was present in the newly formed cells. The coarse cytoplasmic basophilia ascribed to RNA was lost by both the injured and the surviving cells. The latter cells regained this property after the stage of mitosis was completed. At first, the newly formed liver cells were smaller than normal and had a finely granular cytoplasmic basophilia. Later, they showed coarsely clumped basophilic granules. The histological characteristics described at the respective times after injury have been remarkably constant with regard to reproducibility in a group of over 179 rats.

Spectrophotometric—Cytochemical.—The frequency distribution of nuclear classes, according to their respective DNA contents ($2\times$, $4\times$, $8\times$), was markedly altered during the course of the hepatic injury and its repair (Fig. 5). In control group, most of the nuclei were in the $4\times$ class. At 24 hours, when there was no mitotic activity, there was a shift in the nuclear class distribution with more forms intermediate between the $4\times$ and $8\times$ classes. By 48 hours, the most marked changes had occurred. There were no $2\times$ nuclei, there was asymmetry of the distribution of the $4\times$ group, and there were many nuclei of the $8\times$ and higher classes. This stage coincided with maximal mitotic activity. At 72 hours and 120 hours, the nuclear class distributions were still abnormal.

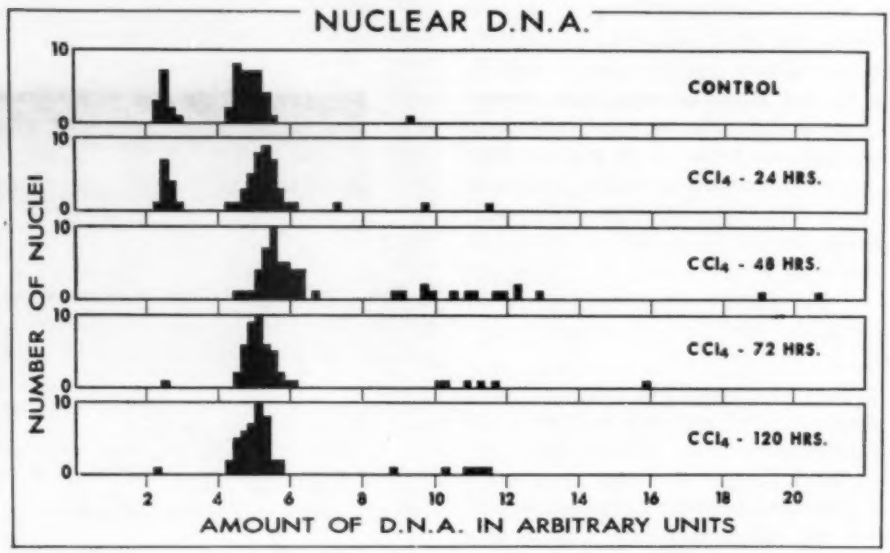

Fig. 5.—Frequency distributions of nuclear classes, based upon the spectrophotometric measurements of Feulgen-stained nuclei, are depicted at various intervals after the administration of CCl_4.

During the course of these studies it became evident that the spectrophotometric method of analysis did not permit complete randomness in the selection of whole nuclei.

Therefore, an additional technique was devised for the analysis of nuclear classes. The number of hits of the three types of nuclei † on the ocular reticle was recorded. These were determined by the study of more than one-third of the nuclei present in the same section which had been analyzed earlier by the spectrophotometric method. The frequency distributions of nuclear classes obtained by these two methods of analysis in a normal rat liver checked very closely (Table 2). The visual method was reproducible in the analysis of replicate sections of the same liver, within 2%, when more than 200 nuclei were counted.

TABLE 2.—*Comparison of Spectrophotometric and Visual Methods of Analysis of Frequency Distribution of Nuclear Classes (Normal Liver)*

Methods of Analysis	Nuclear Classes 2×	4×	8×
Visual (600 nuclei)	.22	.77	.01
Spectrophotometric (100 nuclei)	.21	.78	.01

TABLE 3.—*Frequency Distribution of Nuclear Classes (Per Cent of Group)* *

Groups	Nuclear Classes 2×	4×	8×
Control	.26	.73	.02
24 hr	.23	.73	.04
48 hr	.01	.75	.24
72 hr	.02	.89	.09
120 hr	.01	.86	.13
8 wk	.05	.90	.05

* The percentage distribution for each group was derived from the visual analysis of at least 500 nuclei in each instance.

The shifts in nuclear classes were also evident by this visual method (Table 3). These findings show the marked alterations in nuclear DNA attending injury and repair. The DNA build-up preceded mitosis and the abnormal nuclear classes persisted well beyond the period of mitosis.‡

† The three classes of nuclei are readily differentiated by their differences in size and intensity of staining. The 2 × class of nuclei are the smallest and stain most lightly. The 8 × and higher classes of nuclei are the largest and stain most darkly.

‡ References 14 through 16.

Chemical.—The tissue DNAP and RNAP values (dry weight) rose significantly, at 48 hours after CCl_4, and thereafter fell, ultimately to their starting levels (Table 1). On the other hand, the tissue nitrogen values (dry weight) remained unchanged throughout the experiment. The per cent dry weight of the tissue fell markedly at 48 and 72 hours, so that the nucleic acids and nitrogen contents per unit of wet tissue are appropriately lower at these intervals. The tissue water and lipid contents were not analyzed separately, so the possible contributions of each of these constituents as tissue diluents cannot be estimated. The liver weight rose to a maximum at 72 hours and then fell off. However, even at 120 hours it was still greater than the starting value.

The striking feature of these studies was the marked increase in tissue nucleic acids (dry weight) in contrast to the constancy of tissue nitrogen (dry weight).

COMMENT

This study was planned to define some of the hepatic cell alterations which follow an acute injury. Carbon tetrachloride was chosen as the hepatotoxin because of the simplicity of the experimental design, its reproducibility, and the rapid course of recovery. The histological changes may be considered in three stages: (I) hepatic cell necrosis; (II) inflammation and hepatic cell regeneration, and (III) cytoarchitectural restoration. At Stage I, the tissue nucleic acids and nitrogen levels were unchanged and the frequency distribution of nuclear classes showed beginning shift to higher classes.

At Stage II, there was intense infiltration of the area of necrosis with inflammatory cells and the surviving liver cells showed marked mitotic activity. The liver cells and their nuclei were increased in size. The changes in frequency distribution of nuclear classes were characteristic of growing and regenerating tissues and probably represented a change in nuclear population type as well as the more transient doubling of

nuclear DNA prior to mitosis.§ The tissue nucleic acids were significantly increased during this period of active regeneration. The greater percentage increase in DNAP over RNAP may be ascribed to the amount of DNAP contributed by the inflammatory cells. These are composed chiefly of nuclei and, therefore, heavily weight the changes in DNAP. Thus, the increments in RNAP probably reflected more accurately the regenerative activity of the liver. It is of considerable interest that at the time when the nucleic acid levels (dry weight) were increasing, the protein nitrogen levels (dry weight) remained constant.

Stage III lasted from 72 to 120 hours, longer than either of the earlier stages. The necrotic debris was cleared and replaced by new liver cells. While only rare mitotic figures remained, the shift in nuclear DNA to higher classes persisted. At this time tissue nucleic acid values fell off from earlier higher levels. The lowest fat-free dry weight and the lowest wet-tissue protein nitrogen values occurred at 72 hours.

At 120 hours, histological and chemical restitution were complete, except that the liver size was still increased. Of special interest was the persistence of the abnormal frequency distribution of nuclear classes almost eight weeks after restoration had occurred. Thus, cells were being propagated with nuclei containing more than normal amounts of DNA, as though in preparation for mitosis, but without its occurring. This action suggested the possibility of the development of cells of a different genetic line, with perhaps abnormal growth potential, after only a single major organ injury. That repeated injury with CCl_4 may produce cancer of the liver has been well documented.[17]

Tsuboi and his co-workers [10] have studied this problem in the mouse, given oral CCl_4, and some of their results differ from those reported here. Recalculation of their data from "per liver" to "per unit dry weight and wet weight" showed that an initial fall in nucleic acid levels had taken place during the period of necrosis, with a rise thereafter. This rise was more marked in RNA than in DNA. In the present study, the DNA increment exceeded the RNA increment, and nucleic acid levels did not fall in the early phase of the disease. However, in another set of experiments, which was performed with rats whose livers were later found to be parasitized, liver nucleic acids fell during the progress of CCl_4 injury. Restoration to normal levels occurred when the CCl_4 lesion was healed. These data will be reported in detail elsewhere. The nitrogen data reported by Tsuboi and others were similar to those reported herein. The discrepancies between the results of the present studies and those of Tsuboi and others may be related to species differences between mouse and rat. The data of Tsuboi and others were derived from pooled specimens of liver, and differences between groups of animals cannot be tested statistically. They observed a decrease in liver cholesterol, phospholipid, and neutral fat during regeneration. The water content rose 11% and then decreased. The maximal reduction in fat-free dry weight in their experiments was 22%. Thus, the loss in fat-free dry weight cannot be explained on the basis of the changes in either lipid or water.

Farber and co-workers [11] described a drop in nucleic acid levels at 24 hours after CCl_4, with restoration to normal at 48 hours, in the rat. They commented upon the unreliability of estimating cytoplasmic RNA content from cytoplasmic basophilia.

The present studies show the limitations of each of the analytical methods used and the advantages of the combined use of all of these techniques. The general cytoarchitectural state of the organ is basic information obtained only through histological study. However, the chemical analyses show that the histochemical estimation of RNA from basic dye staining cannot be used as an index of the RNA content of the tissue. At Stage II, when the necrotic and surviving liver cells were free of basophilic clumps, the tissue

§ References 14 through 16.

RNAP was markedly increased. Furthermore, while nuclear DNA changes may be inferred in the presence of large numbers of mitotic figures, significant changes in nuclear DNA with abnormal frequency distribution of nuclear classes may occur in the absence of mitosis. This is readily detectable by the spectrophotometric cytochemical and visual analyses of Feulgen-stained nuclei.

The chemical data give over-all information which cannot be otherwise obtained. Thus, it might be inferred from the histological examination of necrotic livers (24 hours) that a decrease in nucleic acids and nitrogen occurred. However, the chemical data disprove this inference. Also, the marked tissue dilution at 72 hours would not have been detected without the chemical measurements. Lastly, the chemical analyses are good checks on the interpretation of the histochemical estimation of cytoplasmic RNA.

On the other hand, the interpretation of chemical analyses of tissue, without its concomitant histological study, is hazardous. Unless the tissue is homogeneous with regard to cell type, the chemical data lose some of their specific significance and must be considered as over-all values from many cell types.[18] This consideration is especially important in injured tissue where heterogeneity of cell type is marked. It is essential that the chemical data be interpreted in relation to the changing histological picture, else they lose much of their basic pertinence to the entire problem under study.

The results show that the combined use of the histological, cytochemical, and chemical methods offers a more comprehensive picture of the dynamics of hepatic injury and repair than does the use of any one of these techniques separately.

CONCLUSIONS

The responses of the rat liver to an acute injury by carbon tetrachloride have been studied serially. The changes in cytoarchitecture, nuclear DNA, tissue nucleic acids, and nitrogen have been recorded. The results show that anatomical repair is associated with marked increases in tissue nucleic acids, but not in tissue nitrogen. The observed pattern forms a base line for the future investigation of agents which may alter this sequence of events. The limitations of each of the techniques used alone and the advantages of their combined and simultaneous use have been presented.

Dr. Arthur W. Pollister, Professor of Zoology, Columbia University, and Dr. J. Murray Steele, Director, Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, aided in the pursuit of these studies.

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Fine Structure of Renal Glomerulus as Revealed by Electron Microscopy

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The detail of fine structure being revealed in examination of ultrathin sections of tissue by electron microscopy promises to open a new era in the fields of cytology, histology, and histopathology. We have undertaken a systematic study of the fine structure of the kidney by utilizing this technique. Delineation of normal structure is requisite as a base line for evaluation of pathologic alterations which are being studied concurrently. This initial report is concerned with the fine structure of the normal renal glomerulus.

We recently set forth our concept of the structure of the renal glomerulus, which was derived from histochemical and preliminary electron microscopic studies.[1] It is now evident that early electron micrographs were not adequate for a critical view of the delicate organization of this complex structure and that certain interpretations of our own and others require revision. Since 1950 many aspects of the fine organization of the glomerulus have been recorded by pioneers in the technique of histologic examination by electron microscopy. The first report was that of Pease, Baker, and Warren, in 1950.[2] In surprisingly good micrographs for this period, they illustrated certain basic structural features of the glomerular capillaries, including the basement membrane with closely related endothelial cells within and epithelial cells outside the loop. At that time, they considered that a continuous layer of endothelial cytoplasm was not present and noted that "in most places the 'naked' basement membrane is applied directly to the flowing blood stream." They, of course, observed the most striking feature of glomerular structure which has been revealed by electron microscopy, i. e., conspicuous ridges applied to the outer surface of the basement membrane. In a subsequent report by Pease and Baker[3] the opinion was expressed that the surface ridges were an integral part of the basement membrane; they were thought to function primarily as a supporting mechanism. In the same year (1950) Gautier, Bernhard, and Oberling[4] independently described projections lining the outer surface of the glomerular loops. They adduced that projections or fringes of the membrane proper alternated with extensions from epithelial cells which were inserted on the membrane. They described the elaborate arborization and interdigitation of epithelial cell processes and found, "in some fortunate sections," the endothelial membrane "showing itself as an extremely fine pellicle reinforced by a reticulum of rather regular design effecting the appearance of a honeycomb."

Dalton,[5] in 1951, considered the ridges applied to the basement membrane to be processes of the epithelial cells and also described a very delicate endothelial membrane internal to the basement membrane. Thus, he found three membranes to be interposed between the blood stream and the lumen of Bowman's capsule: a complete endothelial lining, a continuous basement membrane, and a layer formed by the fine interdigitating processes

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of the cells of the visceral layer of Bowman's capsule.

In 1953 we recorded preliminary electron microscopic observations of the normal glomerulus in conjunction with a histochemical study of both normal and pathologic glomeruli.[1] Our observations indicated that the basement membrane proper was a derivative of the endothelial cells. We also noted a "latticelike porosity," which we thought at that time to be a fenestration of the basement membrane.

Hall has recorded his observations on the fine structure of the glomerulus in several reports.* He described the "basement membrane" of the glomerular capillaries as a "complex of three intimately related specialized structures (1) the luminal, delicate, highly porous submicroscopic layer, the 'lamina fenestra' (lining network), (2) a thin, smooth, dense but probably finely porous membrane which must serve as an ultrafilter, the 'lamina densa' and (3) an intricate external layer composed largely of characteristically shaped, rather uniform processes, the 'pedicles,' which are terminal extensions of secondary processes, trabeculae, from 'podocyte' (epithelial) cytoplasm." He found the foot processes slightly separated from his "lamina densa," i. e., the true basement membrane, and found the foot processes variously separated from each other, leaving narrow strips of the basement membrane exposed directly to the space of Bowman.

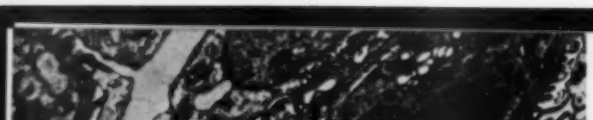
In Pease's most recent study of the glomerulus [10] he described terminal processes of epithelial cells attached to the basement membrane and observed consistent, rather uniform spaces between such processes. He noted that portions of the cytoplasm of the endothelial cells were extremely attenuated and, like Hall,[7] observed a regularly arranged system of "pores" throughout the endothelial sheet. These "pores" were of nearly constant diameter (approximately 0.1μ). However, he was uncertain whether or not the pores were genuine or fixation artifacts, "for in life a thin film of watery cytoplasm might span these gaps."

In a recent brief report of glomerular structure as revealed by electron microscopy, Reid [11] described the basement membrane of the glomerulus as a definite layer which was different in density from the cytoplasm of adjoining endothelial and epithelial cells. The entire external surface of the basement membrane appeared to be covered with cytoplasm of the epithelial cells. He noted that vacuoles were common in the cytoplasm of endothelial and epithelial cells and suggested that the honeycomb appearance of the endothelium, described by Oberling, Gautier, and Bernhard,[12]—Hall's[7] "lamina fenestra"—may represent a degenerative change in the endothelial cytoplasm. He found no evidence of penetration of the basement membrane by epithelial cytoplasm, which we had suggested.[1]

Our current studies lend support to some of the observations cited, while negating others, and should serve to extend our understanding of fine glomerular organization and to clarify some of the present points of question.

To those of us who have spent many years viewing tissues by light microscopy—utilizing even the most exacting techniques—it is indeed a surprise, and a refreshing one, to view the exquisite detail of cell structure and organization that can be seen in good electron micrographs. Indeed, the fine structure of cells and of tissue organization being revealed affords an exciting structural basis for function. The physiologist and anatomist now have an opportunity to correlate fine cell structure with function, and the experimental pathologist has a sensitive method for study of the intimate sites of and reaction to injury. It is our hope, after presenting this study of the glomerulus, to examine the fine structure and organization of the tubules, blood vessels, and supporting elements of the normal kidney as a basis for investigation of alterations accompanying physiological stresses and injuries.

* References 6 through 9.



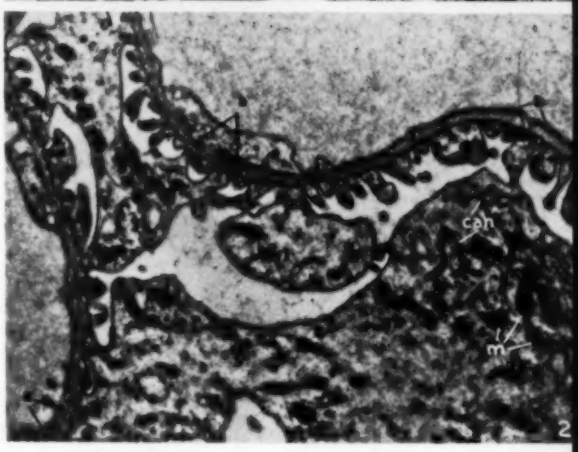


Fig. 1.—This relatively low-power electron micrograph shows the general pattern of organization of glomerular loops. Segments of two loops are shown. Endothelial cells with relatively dense nuclei lie within the loops. The cells show cytoplasmic branches applied to the basement membrane proper. It will be seen that a cytoplasmic layer of irregular thickness lines the inner aspect of the rather sharply defined basement membrane. At this magnification the thin segments of lining endothelium are scarcely visible. At the outer aspect of the basement membrane are the large epithelial cells with elaborately branching cytoplasmic processes. Nuclei of three epithelial cells and cytoplasmic segments of other such cells are included in the field. It will be seen that foot processes of the epithelial cells are inserted over the entire outer surface of the basement membrane. Reduced ⅓ from mag. × 5000.

Fig. 2.—Detail of the basement membrane proper, with adjoining endothelium and epithelium. Orientation is readily made, if it is recalled that the characteristic foot processes are derivatives of the epithelium and so serve to localize the outer aspect of the glomerular loop. Below is a large segment of epithelial cytoplasm, and a smaller segment is present at the upper left. It will be seen that a number of the foot processes are directly traceable to the larger segment of epithelial cytoplasm. The epithelial cytoplasm shows a fine, light, nearly homogeneous background. It contains numerous dark mitochondria (*m*) and scattered canalicular structures (*can*) of the endoplasmic reticulum with the closely associated fine cytoplasmic particles. The cytoplasm also contains a number of vesicles of various sizes. The foot processes completely surface the outer aspect of the basement membrane to which they are attached. Many of the foot processes present a hoof-like form. It will be noted that they lie in essential apposition to one another. Endothelial cytoplasm completely lines the inner aspect of the portions of two capillary loops shown. It is of variable thickness. The thicker segments contain a number of vesicles of irregular size. The thinner segments of endothelial cytoplasm seen to the right above and to the left below contain chains of fine vesicles (*a*). In the zones marked "*b*", the fine line of density between the basement membrane and the foot processes is absent and is replaced by a line of exquisitely fine vesicles.

The basement membrane proper is not fully homogeneous but appears very finely porous in the original photograph. Reduced ⅓ from mag. × 16,500.

MATERIALS AND METHODS

Small blocks of kidney from deeply anesthetized, normal rats were fixed immediately. Thin slices of the renal cortex, cut with a razor blade, were placed in shallow dishes containing 1% buffered osmic acid adjusted to pH 7.4.[13] While immersed in the fixative, the slices were then cut into pieces of approximately 1 cu. mm. The blocks were fixed for two hours. After fixation the tissue was rinsed in two or three changes of distilled water and dehydrated in graded alcohols over a total period of three hours. The short fixation and rapid dehydration were suggested by Palade.† After dehydration the tissue was infiltrated and embedded in *n*-butyl methacrylate. Sections in the range of 0.1μ to 0.025μ were cut with a glass knife, using the Servall microtome (adapted after the design of Porter and Blum [14]).

The sections were examined, without removal of the embedding medium, in an RCA EMU electron microscope, using a wide field, objective pole piece. Selected fields were photographed at initial magnifications of × 600 to × 5000 and enlarged photographically, as desired. The principal factors which we believe to be responsible for improved tissue preservation are the use of buffered osmic acid and short fixation followed by rapid dehydration. The microtome used permits well-controlled thin sectioning.

† Palade, G. E.: Personal communication.

OBSERVATIONS

There are three chief components of the glomerulus, the endothelium, the basement membrane, and the surfacing epithelium. The endothelial cells are about three times as numerous as the epithelial cells.[7] Both the endothelial and epithelial cells show highly specialized and variously differentiated cytoplasm. The endothelial cells have ovoid nuclei which are somewhat smaller and denser than those of the epithelial cells (Fig. 1). Most of the cytoplasm of the endothelium is of rela-

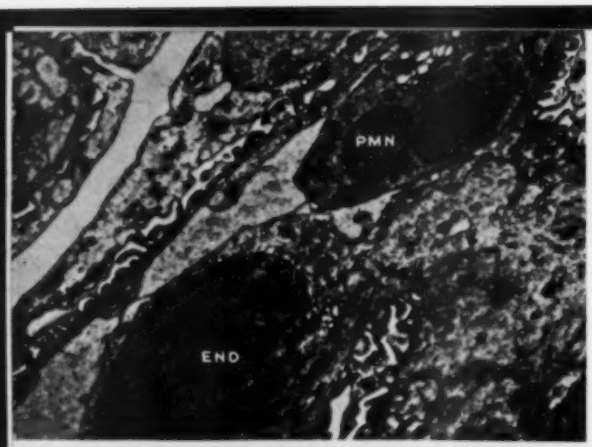


Fig. 3.—This photograph shows detail of a capillary loop. Below is an endothelial cell nucleus (*END*). In the right lower quadrant of the photograph the endothelial cytoplasm has been cut in a tangential plane. It will be seen that strands extend from the endothelial cytoplasm to merge with the basement membrane (arrows). This constitutes one of the evidences which lead us to believe that the basement membrane is a derivative of the endothelium. It is probably a differentiated product of the marginal cytoplasm. It will be observed that the densities of the cytoplasmic strands are quite the same as those of the membrane proper. A polymorphonuclear leucocyte (*PMN*) fills the extremity of the capillary loop shown above. The leucocyte contains a vacuolar Golgi zone, several mitochondria, and a number of rounded cytoplasmic granules. Reduced ⅜ from mag. × 12,000.

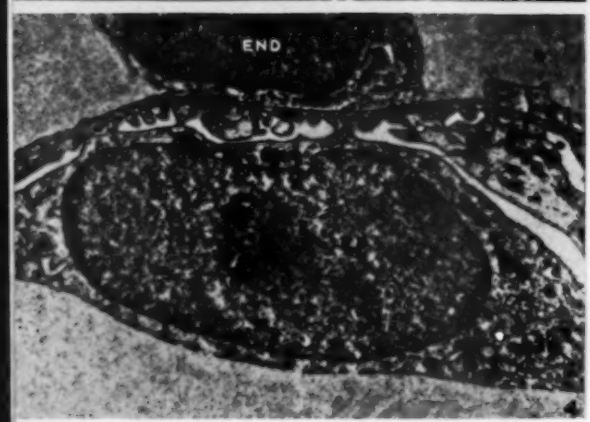


Fig. 4.—A short segment of a glomerular capillary loop is shown in detail, with a typical epithelial cell below and endothelial cell above. The exquisitely fine granular texture of the epithelial cell nucleus is well shown. It contains two nucleoli. The surface of the nucleus is bordered by a fine "double membrane." Several mitochondria are visible in the cytoplasm of this cell, and it contains fine canalicular structures of the endoplasmic reticulum (*can*) which are bordered by minute particles. The cytoplasm also contains numerous small vesicles. It will be noted that mitochondria are present even in peripheral branches of the epithelial cytoplasm. Several of the foot processes can be traced directly to the larger cytoplasmic mass. It will be seen that foot processes completely surface the outer segment of the basement membrane. Small tent-like clear spaces are seen, bordered by the basement membrane above and the margins of the foot processes at the sides. The basement membrane proper is clearly outlined by the borders of the foot processes below and the border of the endothelial cytoplasm above. It is of essentially uniform thickness. An endothelial cell lies just above the basement membrane. It contains cytoplasmic constituents similar to those of the epithelial cell. The nucleus is denser than that of the epithelial cell. The nuclear particulates appear to be of similar size in the two cells, but those of the endothelial cell appear more closely aggregated. The lining layer of endothelial cytoplasm is well shown. At the left, the thin segment of endothelial cytoplasm appears to show occasional apparent interruptions. These are thought to be "more seeming than real," as indicated in the text. Reduced ⅜ from mag. × 13,500.

tively light density and possesses the usual cellular components, i. e., mitochondria, small vesicles and occasional "canalicular" structures of the endoplasmic reticulum (ergastoplasm), and the associated fine cytoplasmic granules described by Palade [15] (Figs. 3, 4, and 5). In satisfactory preparations, the internal structure and borders of the delicate endothelial cytoplasm are well preserved. Some sections reveal branching processes of the endothelial cytoplasm (Figs. 1 and 5). Except for occasional small questionable interruptions, the endothelial cytoplasm completely lines the inner aspect of the basement membrane. The endothelial cytoplasm is of variable and irregular thickness. In some places this layer is quite thin. In its thinner segments it often contains a series of small vesicles in the range of 200 A. to 800 A. in diameter (Figs. 2, 3, 4, and 5). In some sections there are scattered short segments, where the attenuated endothelial cytoplasm appears to be interrupted. However, these probably represent either small segments of cytoplasm devoid of fluid or cytoplasmic vesicles where the surface border is not visible. Usually a fine line of density is seen between the endothelial cytoplasm and the basement membrane. However, in some sites this is lacking and is replaced by a row of very fine vesicles (*c*, in Figs. 5 and 6).

A distinct membrane of relatively uniform medium density, which averages approximately 800 A. in diameter, separates the endothelium on the inside of the glomerular loop from the epithelium on the outside. This is the basement membrane proper. Careful

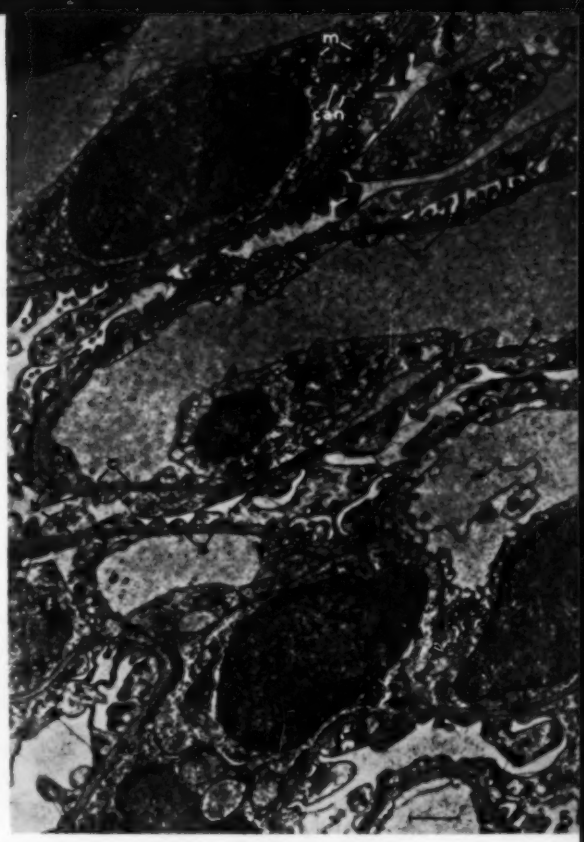


Fig. 5.—This photograph shows particularly good detail of fine structure. Segments of four endothelial cell nuclei are present and serve to localize the interior of the glomerular loops. No epithelial cell nuclei are present, but the elaborate branching epithelial cytoplasm is well shown. The endothelial cell, above, shows a prominent nucleolus in a somewhat folded nucleus of a finely granular texture. In the surrounding cytoplasm mitochondria with well-defined "cristae" are clearly seen (*m*). The cytoplasm contains many small vesicles. A few strands of the canalicular endoplasmic reticulum are visible (*can*). The branching character of the endothelial cytoplasm is evident, and it may be seen that a layer of endothelial cytoplasm completely surfaces the inner aspect of the basement membrane. In some of the thin segments it contains rows of round or ovoid vesicles (*a*). It will be noted, however, that this feature is evident only in certain segments of the cytoplasm and clearly absent in other portions.

The basement membrane proper is very clearly shown. It averages approximately 800 A. in thickness. It is of a relatively uniform, gray density. However, on careful inspection of the original photographs it is not fully homogeneous but presents a very fine porous appearance.

In this photograph, detail of the epithelial cytoplasm is particularly well preserved. Foot processes of the epithelial cells, which are of varied dimensions, fully surface the outside of the basement membrane. They appear directly to adjoin one another laterally. The epithelial cytoplasm exhibits a fine "dust-like" density and contains scattered, relatively small mitochondria and numerous vesicles of varied size. The foot processes, in general, are clearly demarcated from the basement membrane by a very fine line of sharp density. However, in some sites this line of density is lacking and is replaced by a series of small vesicles in the bordering margin of the process (*b*). This appearance strongly suggests transfer of fluid from the basement membrane into the foot processes. A similar appearance is noted in certain junctures of the endothelial cytoplasm and basement membrane. Here again, while there is ordinarily a fine line of density between the delicate endothelial cytoplasm and the basement membrane, in some situations the two layers appear to merge and a series of very fine vesicles may be seen (*c*). Certain of the finest structural detail noted can scarcely be reproduced and is shown in the subsequent Figure, in which the detail of vesiculation is accentuated with dots of white ink. Reduced ⅜ from mag. × 12,000.

inspection of many micrographs, as well as previously cited observations,[1] offer strong, if not fully convincing, evidence that the basement membrane is a derivative of the endothelial cytoplasm. In histochemical studies previously reported it was found that at least some of the "endothelial cytoplasm" surrounding the nuclei stains like that of the "basement membrane." In some electron micrographs strands of "differentiated" endothelial cytoplasm are seen to contribute directly to the constitution of this membrane (Figs. 3 and 6). In places the endothelial cytoplasm appears to merge almost imperceptibly with the basement membrane, while the demarcation between the epithelium and the basement membrane is usually distinct.

Fine Structure of Basement Membrane.—In evaluating the finest elements of structure, one must be cautious to avoid misinterpretations due to artifacts. Certainly, in many segments of the basement membrane it is not possible to discern structural differentiation. However, in some sections the basement membrane appears to show a fine structural organization. This is seen as an exquisitely delicate vacuolar or porous structure, so fine that it is scarcely reproducible (Figs. 2 through 6 and 7).

While the basement membrane is undoubtedly a semipermeable membrane from the evidence at hand, it does not appear possible to assign to it a precise, measurable, internal structure. In some sections a band of less density of the membrane is noted in the zone bordering the epithelium (Fig. 7). This change in density may be related to fluid transfer and reflect a greater fluid content in this area.

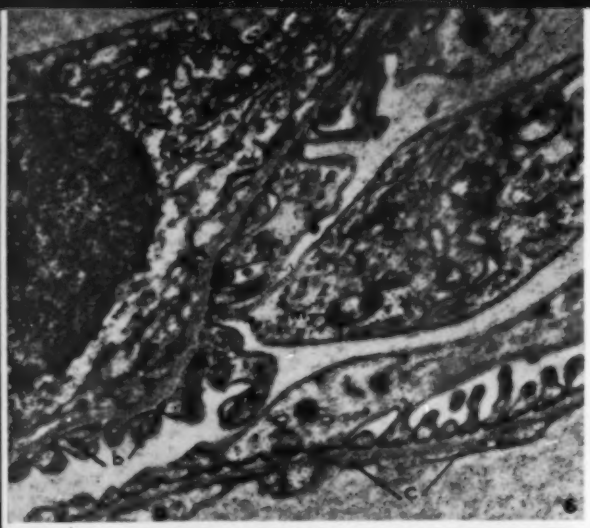


Fig. 6.—The upper part of the field shown in Figure 5 has been further enlarged in this illustration. A portion of an endothelial cell is present at the left. The nucleus is bordered by a "double membrane." Several mitochondria (*m*) with well-defined "shelf-like" cristae are present in the cytoplasm, as well as canalicular structures (*can*), tiny cytoplasmic particulates, and small vesicles. The very fine vesiculation of portions of the endothelial and epithelial cytoplasm and rows of fine vesicles bordering the inner (*c*) and outer (*b*) aspects of the basement membrane (which are scarcely reproducible) have been dotted with white ink to illustrate their distribution, as described in the text. Careful inspection reveals a very fine "porosity" of the basement membrane proper, which has not been marked. Reduced ⅜ from mag. × 24,000.

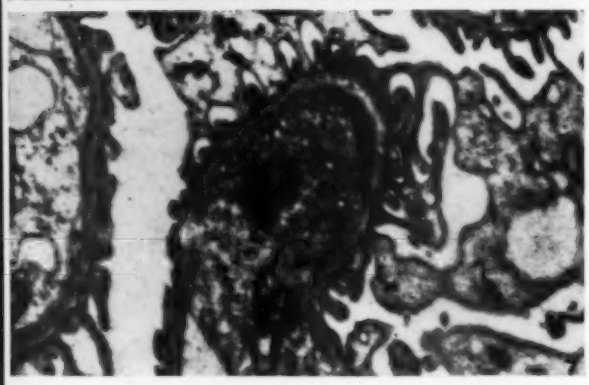


Fig. 7.—This Figure shows the tip of a capillary loop. A number of vesicles are seen in the endothelial cytoplasm, which has been cut tangentially. It is evident that there is no regularly arranged fenestrated membrane. The basement membrane has been cut tangentially also. It will be noted that its outer zone is of less density than the inner part. Though less marked, this phenomenon may be seen in other sections (note the small segment of a capillary loop at the upper right). The elaborate arborization of the epithelium is well illustrated. The increased density of the foot processes in this photograph is due to the somewhat greater thickness of the section. Reduced ⅜ from mag. × 21,000.

Glomerular Epithelium.—The organization of the epithelium surfacing the capillary loops is even more elaborate than is that of the endothelium. This is the most remarkable structural feature revealed by electron microscopy and had only vaguely been seen before (von Möllendorff).[16] The epithelial cells exhibit most complex cytoplasmic "arborizations." We find it particularly difficult to visualize this organization in three dimensions. However, it appears that the epithelial cells have "innumerable" branching processes which are ultimately anchored upon the basement membrane. The epithelial cells possess relatively large, ovoid nuclei which are somewhat less dense than those of the endothelial cells. They are surrounded by a relatively pale cytoplasm containing the fine structural elements found in most cells, i. e., mitochondria, tiny but various-sized vesicles as well as occasional "canalicular" structures of the endoplasmic reticulum, and the associated fine cytoplasmic granules. As in other cells, the fine cytoplasmic granules are found immediately adjoining the "canalicular" structures of the endoplasmic reticulum (Figs. 2, 4, and 6). The background of the epithelial cell cytoplasm presents a fine, dust-like amorphous appearance and contains numerous vesicles. The cytoplasm of the "primary" branches of the epithelial cells is similar to that which surrounds the nucleus and commonly contains mitochondria. The peripheral cytoplasmic fronds of the epithelial cells in general exhibit a somewhat greater density than that of the remaining epithelial cytoplasm. However, this is not always so. The foot-like processes attached to the basement membrane may exhibit a cytoplasm of low density. The narrower segments of the branching epithelial cytoplasm are denser than the broader portions. The epithelial pegs or foot processes attached to the basement membrane commonly have a pyramidal form resembling a horse's hoof. The marginal segments of the epithelial cytoplasm, particularly when cut tangentially, show a very delicate "sponge-like" structure, in effect, a close aggregation of tiny vesicles bordered by fine lines of density (Figs. 2 to 5, and 6).

For the most part, "the epithelial pegs" abutting the "basement membrane" are sharply demarcated from it by a fine line of sharp density. However, in some sites this sharp line of demarcation is not present, and a row of fine vesicles about 100 to 200 A. in diameter lie at the margin of the epithelial cytoplasm of the foot processes adjoining the basement membrane (*b*, in Figs. 2, 5, and 6). At times, somewhat broader masses of epithelial cytoplasm are attached to the basement membrane. The "sucker feet" of the epithelial cytoplasm applied to the basement membrane are approximated in most, if not all, areas. Tiny, tent-like clear spaces are commonly seen, bordered by the basement membrane and the lateral margins of the foot processes (Figs. 2, 4, 5, and 6). It is our belief that most, if not all, of the foot processes abut upon the ones adjoining just above the basement membrane.

The full glomerular surface is covered with the foot processes or somewhat broader segments of epithelial cytoplasm. The occasional sites where they appear to be lacking probably represent sections cut in a plane directly between adjoining processes. It is also evident that processes from one epithelial cell may be anchored to the basement membrane of two or more capillary loops. Further, it appears that each epithelial cell and its many processes effectively "hood" certain segments of the capillary loops. Consequently, fluid traversing the endothelial cytoplasm, its basement membrane, and the foot processes of epithelial cells is exposed to an elaborate series of "baffles" of epithelial cytoplasm before it passes into the tubular system.

COMMENT

While the general organization of the glomerular loop has been revealed by the prior electron microscopic studies cited, it is clear that much of the detail of structure reported here has not been described before, and some of the earlier observations were made on overfixed or imperfectly fixed tissue, with resultant shrinkage and loss of fine cytoplasmic structure. The fine detail of structural organization has a very important bearing on any functional interpretations derived from it.

From the observations recorded, it is evident that there are three distinct layers between the blood stream and the Bowman space. The inner component consists of lining endothelial cells which present considerable amounts of delicate cytoplasm containing the usual cytoplasmic constituents. Not infrequently, such cells show branching cytoplasmic processes inserting at several sites along the basement membrane proper. A layer of endothelial cytoplasm completely lines the inner aspect of the basement membrane. The occasional apparent interruptions in thin segments of this layer are probably not real. In the thinner segments the cytoplasm is about 200 to 400 A. in diameter. It is probable that distortion of vesicle-containing cytoplasm, incident to overextraction of tissue, has produced the "honeycomb" lattice of Gautier and co-workers [4] or the "lamina fenestra" of Hall.[7] It should be pointed out that this relatively coarse vesiculation of the thin endothelial cytoplasm is seen only in certain segments and is clearly lacking in others. It seems, then, that this layer of endothelium does not present a fixed porosity or fenestrated structure, as suggested by Hall.[7] Also, we must admit our own error; we find no evidence in improved sections that there is a periodic penetration of the mucinous cytoplasm through gross pores in the basement membrane as previously suggested by us.‡

The second layer of the glomerular loop is the basement membrane proper. This appears to be very finely porous or sponge-like. It does not seem possible, at this time, to secure

‡ Reference 1. However, since this report was submitted for publication, we have observed that the cytoplasm of some epithelial cell foot processes fuses with and appears to permeate the basement membrane. Pads of a substance of similar density to that of the foot processes are found on the endothelial side of the basement membranes directly opposite the foot processes. Zones of such irregularly distributed mucinous substance *inside* the basement membrane might account for the occasional appearance of coarse fenestration in this position.

sufficiently fine resolution to assign a precise dimension to this porosity (if it is, indeed, uniform). It would, however, appear safe to say that it is less than 100 A. For the reasons previously set forth, the basement membrane is considered to be primarily a differentiated cytoplasmic product of the endothelium. The basement membrane of the glomerulus differs somewhat from other basement membranes. Most basement membranes stain as reticulin, with appropriate silver impregnation techniques. The glomerular basement membrane does not stain in this manner. The reticulin-staining property is thought to be due to the combined presence of fine collagen fibrils in a "gel-like" matrix of glycoprotein.§ We have not found evidence either from electron micrographs or from histochemical studies that collagen fibrils are present in the normal glomerulus.

At the outer aspect of the glomerular loops are the elaborately branched epithelial cells, with "innumerable" foot processes which fully cover the basement membrane. This constitutes the third layer of the glomerular loop. In the angles formed by the basement membrane and adjoining foot processes, minute tent-like spaces of less density may be seen. Most, if not all, of the foot processes abut upon the ones adjoining them just above the basement membrane. Although some appear to be very slightly separated, it is believed that the delicate spongy cytoplasm of adjoining foot processes are in contact. This observation is contrary to that of Hall [7] and Pease.[10] In general, the border between the basement membrane and the inserted foot processes of the epithelial cells is sharply demarcated by a very fine dark line. However, in some sites this sharp line of demarcation is not evident and is replaced by a row of fine vesicles in the cytoplasm of the foot processes bordering the basement membrane (*b*, in Figs. 2, 5, and 6). The vesicles here are exquisitely delicate, being in the range of 100 to 200 A. in diameter.

§ References 17 and 18.

It is, of course, tempting to speculate upon the functional significance of this elaborate and delicate organization. Additional studies of the fine structure of the glomerulus under physiologic stresses should aid in such an evaluation. However, the structure described does suggest certain functional mechanism, which will be set forth briefly and tentatively.

If, as it appears, the endothelial cytoplasm completely layers the inner surface of the basement membrane, this layer affords the first unit to be traversed in the elaboration of the glomerular filtrate. The surface layers of endothelial cytoplasm commonly present a fine sponge-like structure. The frequent finding of a chain of small vesicles in the attenuated endothelial membrane suggests that minute plasma droplets are "taken up" by it. Also, the prominence of vesicles in the broad segments of endothelial cytoplasm and the observation that the under border of somewhat thickened segments of endothelial cytoplasm is found, in some sites, to be replaced by a series of very fine vesicles further indicate fluid transfer across the endothelium to the basement membrane via fine vesicles.‖ Such vesicles would then be exposed to ultrafine porosity of the basement membrane proper, which would serve to remove most, if not all, of the protein. The fluid then would meet an epithelial layer before being passed into the Bowman space. One avenue of escape would seem to be into the minute tent-like spaces where the foot processes adjoin. Even here it seems that the fluid would have to pass through epithelium. The frequent finding of fine vesicles in the cytoplasm of the foot processes, where they border the basement membrane, suggests a direct transfer of fluid from the basement membrane into the cytoplasm of the epithelial cells, where it appears as fine vesicles.

In view of the elaborate and highly specialized organization of the visceral epithelium

‖ Palade [19] first suggested that fluid might be transferred across endothelial cytoplasm via vesicles in the study of capillary endothelium of skeletal muscle, heart muscle, pancreas, and intestine; we have found support for this view in studies of the sinusoids of the anterior pituitary gland.[18]

of the glomerulus, it is scarcely conceivable that it does not play an active role in glomerular filtration. If, as histochemical studies suggest,[1] the cytoplasm of the epithelium is in the nature of a mucopolysaccharide or mucoprotein, it may exert a tangible filtration force due to its hydrophilic property. Fluid might then be passed into the Bowman space by diffusion through the epithelial cytoplasm. Nothing is known of the mobility or possible contractility of the visceral epithelium of the glomerulus. It is possible that this cell may possess a degree of contractile power and free itself of excess fluid by a process analogous to squeezing a sponge.¶ (The fine sponge-like organization of surface segments of the epithelial cytoplasm has been noted.) This would discharge the "filtrate" into the Bowman space and, perhaps, exert a certain propulsive force upon it, as it passes into the proximal tubules. The "dehydrated" epithelial sponges could again act in elaboration of further filtrate. Even when the fluid filtrate has passed these fine barriers, it would be exposed to an elaborate baffle of epithelial cytoplasm en route to its perhaps even more exciting excursion through the renal tubules.

SUMMARY

Electron microscopy has afforded a new view of the fine structure of the normal glomerulus. The electron microscopic studies of the glomerulus reported since the first observation in 1950 are briefly reviewed. While these studies have revealed the essential features of glomerular organization, technical improvements in fixation and sectioning which have been developed in the past year permit better preservation of fine cytologic detail.

In this report the fine organization of the glomerular loops is described and illustrated and certain physiologic implications are briefly discussed. Three elements enter into the composition of the glomerular loops, namely, an inner layer of endothelium, a basement membrane, and an outer layer or elaborately organized epithelium. The lining layer of endothelium is complete and of variable thickness. The attenuated segments of endothelium are in the range of 400 A. in thickness. This layer may contain a series of vesicles, giving it a pseudoporous appearance. The thicker segments of endothelium cytoplasm also contain many small vesicles. The basement membrane proper is a structure about 800 A. in thickness. It appears to possess an extremely fine porosity. Evidence is presented that the basement membrane is primarily a derivative of the endothelial cell.

The elaborate organization of the surface epithelium is the most remarkable feature of fine structure revealed by the electron microscope and, undoubtedly, importantly related to function. The epithelial cells show branching cytoplasm with terminal foot processes which are anchored to and fully surface the outer aspect of the basement membrane.

A series of observations are presented which strongly suggest that fluid is transferred across the endothelial cytoplasm via small vesicles. The basement membrane, if very finely porous, would serve as an effective filter for removal of protein. The elaborate organization of the epithelium strongly suggests that it plays an active role in preparation of the glomerular filtrate. Evidence is presented that fluid, having traversed the basement membrane, appears at the adjoining margin of epithelial cytoplasm as a series of fine vesicles. The "sponge-like" character of portions of the epithelial cytoplasm is described. It is suggested that the epithelium may be hydrophilic and possess contractile properties and, so, function somewhat like a contractile sponge as a part of the complex mechanism concerned with the elaboration of the glomerular filtrate.

Robert Brooks and Mrs. Karin Taylor gave technical assistance in this study.

¶ McManus [20] has recently postulated a myoid nature of the cells covering the renal glomerulus. This idea was based chiefly upon the demonstration in this epithelium of the enzyme 5-nucleotidase, which is present characteristically in smooth muscle cells.

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Multiple Arteriosclerotic Aneurysms of Coronary Arteries

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Although the all too common lesion of coronary arteriosclerosis not infrequently reaches severe proportions from the fifth decade onward, the development of an arteriosclerotic aneurysm of the coronary arteries is interestingly rare. Mitchell [1] in 1947 collected 17 cases from the world literature and reported 1 of his own. Sarkisian [2] in 1950 added another, making a total of 19. Multiple arteriosclerotic aneurysms involving both major branches of the coronary arteries are even rarer. The first case reported was that described by Mitchell.[1] The second will be described here.

Of more interest than the rarity of this lesion, however, is a possible explanation of the pathogenesis of this pathological oddity that was suggested by the examination of the material prepared from this case.

REPORT OF A CASE

This 58-year-old white married man was admitted to the State Hospital, at Raleigh, N. C., in 1950, with symptoms of schizophrenia, paranoid type. At the time of admission there was a history of having had a "stroke" two years previously, with a resulting left hemiparesis. Physical examination revealed generalized arteriosclerosis, with a blood pressure reading of 190/130 mm. Hg. The heart was "enlarged to the left." The left hemiplegia was noted, and there were associated pyramidal signs in the left extremity. The patient was confined to his bed throughout his hospital stay, with no essential change in his psychiatric status. His physical condition remained satisfactory, although repeated blood pressure readings maintained levels approximating 200/120 mm. Hg. On Aug. 10, 1953, he suddenly experienced a bout of acute pulmonary edema. His temperature was 100.4 F, pulse weak and rapid, respirations extremely labored, and blood pressure 170/80 mm. Hg. Examination of the chest at this time revealed many coarse rales and rhonchi. The heart sounds were poorly heard. X-rays of the chest showed large "densities" in the central lung fields. The transverse diameter of the heart was 18 cm. and the aorta appeared to be rather tortuous. His condition deteriorated steadily. In spite of a wide range of antibiotic therapy his fever did not subside. The cardiac status remained refractile to digitalis and diuretics. He lapsed into coma and died on Aug. 13. Laboratory findings showed a normal hemogram and blood sugar. There was no evidence of syphilitic infection. During his terminal episode his NPN rose to 88 mg. per 100 cc. The final clinical diagnosis was hypertensive cardiovascular disease with terminal uremia and bronchopneumonia.

Autopsy Findings.—Autopsy (No. AS-53-26) was performed by Dr. Daniel Beals five hours post mortem, and the significant pathological findings were referable to the cardiovascular system. The body was that of a well-developed, slender, elderly white man. A bilateral hydrothorax of about 300 cc. was found. The pericardial sac contained about 60 cc. of amber fluid.

Heart.—The pericardial surface was not remarkable. The transcardiac diameter in situ measured 13 cm. The heart weighed 650 gm., and its epicardial surface was smooth and glistening. The left mural myocardium was 1.7 cm. thick at the attachment of the anterior papillary muscle. The right ventricular wall measured 0.3 cm. in thickness. The myocardium was firm, was dark red in color, and showed diffuse fibrosis but no recent infarcts.

There were five bulbous aneurysmal dilatations involving both of the tortuous coronary arteries. The largest measured 3 by 3 by 2.5 cm. and was found in the right coronary artery 6 cm. from its origin. Two centimeters distal to this was a second, smaller, dilatation, measuring 3 by 1.5 by 1.7 cm. Just beyond this was a third and smaller defect, measuring 2 by 1.4 by 1 cm.

The left coronary artery divided almost immediately into its anterior descending and circumflex branches. At the origin of the descending branch a fourth aneurysm was found that measured 2.5 by 1.5 by 1.5 cm. Three separate and distinct

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vessels emerged from this dilatation. The central one followed the normal route of the left anterior descending coronary. A medial branch of equal diameter penetrated the myocardium and traveled toward the junction of the right and left ventricles. The lateral branch passed over the outer surface of the left ventricle.

Along the course of the circumflex branch of the left coronary artery, 1 cm. from its origin, was a fifth saccular aneurysm, measuring 3.5 by 2.5 by 2.4 cm. Just distal to this was a terminal branch that turned sharply and traversed the surface of the left ventricle toward the apex.

Cut sections of all of the aneurysmal dilatations appeared essentially the same. Each contained numerous layers of laminated blood clot that left

Fig. 1.—External view of the heart showing the cut surface of one of the larger aneurysms of the right coronary artery with its laminated thrombus and residual lumen.

only a residual lumen. Figure 1 shows representative cross sections. A muscularis was not visible grossly within the vessel wall. Small foci of calcium were found and there was no evidence of leakage or frank rupture.

The root of the aorta was slightly dilated and contained moderately severe atherosclerotic plaques. The coronary ostia were of a normal diameter. The circumferences of the valves were as follows: tricuspid, 13 cm.; pulmonary, 8.5 cm.; mitral, 10 cm., and aortic, 8 cm. The valve leaflets were filmy and competent. The right lung weighed 870 gm.; the left lung, 770 gm. Both were markedly edematous. All the abdominal organs showed evidence of chronic passive congestion.

No evidence of severe infection, syphilis, or source of embolic phenomena that might otherwise serve to explain the development of the aneurysms was found.

The microscopic studies of the sections of the aneurysms are described at some length below. Those of the remaining organs were not remarkable.

COMMENT

Although the differences put forward in the three prominent concepts as to the pathogenesis of arteriosclerosis * have not been resolved, most authors agree that the initial and severest changes take place primarily in the intima. These have been variously described as deposition of droplets of fat, proliferation of fibroblasts, appearance of crystals of cholesterol and cholesterol esters, fragmentation of the elastica, liquefaction of the lipid, hemorrhage, calcification, and, not infrequently, ulceration and thrombosis.

Moore [6] states: "In the coronary arteries there is atrophy of the media beneath a plaque, but not direct extension [into it]." As is described below, this case, however, showed focal destruction of the media before it was actually compressed by the expanding intimal lesion.

Like all of the so-called muscular arteries, the coronary arteries contain only minimal amounts of elastic tissue. Unlike the aorta, then, the resistance to dilatation and perforation is offered by the large muscular component of the media rather than the elastica. In spite of the lengthy descriptions that have been written concerning the fragmentation and destruction of the elastic fibers in these particular vessels, they probably play a very minor part in arteriosclerosis.

All of the sections taken from the several aneurysms and many sections taken from the coronary arteries elsewhere showed the typical subintimal lesion of fat deposition, fibrosis, and hemorrhage. In each of the aneurysmal areas ulceration of the endothelium had occurred with thrombus formation.

Of greater interest, however, was the study of the media and adventitia. The muscularis was generally compressed in the path of the expanding intimal lesion. Masson's stain

* References 3 through 5.

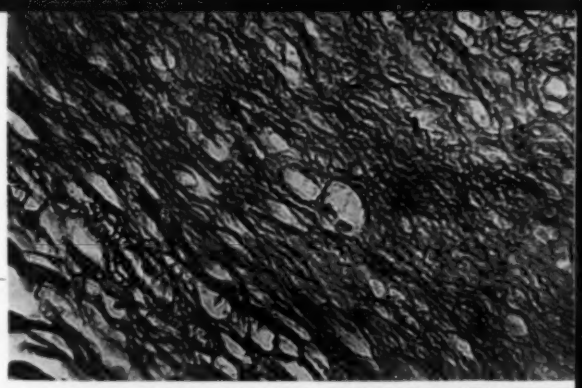

Fig. 2.—High-power view of a section of the wall of one of the smaller aneurysms to show the cystic changes within the muscle of the media. The lesion at this stage is free of inflammation. Masson's trichrome stain; reduced ⅕ from mag. × 100.

revealed the fibers to be intact in some areas, paler and more cystic in others, and the seat of sharp dissolution in still others. The cystic spaces appeared to contain edema fluid rather than a mucoid material (Fig. 2). In some of the disrupted areas the adjacent muscle layers showed fragmented ends but retained much of its normal thickness (Fig. 3). At the locus of disruption it was replaced by a network of connective tissue, in whose interstices were seen numerous red and white blood cells. Many of the spaces were not lined by endothelium and could not be considered orthodox blood channels. Monocytes and lymphocytes were present in varying numbers, and blood break-down pigment was found within and without macrophages (Fig. 4).

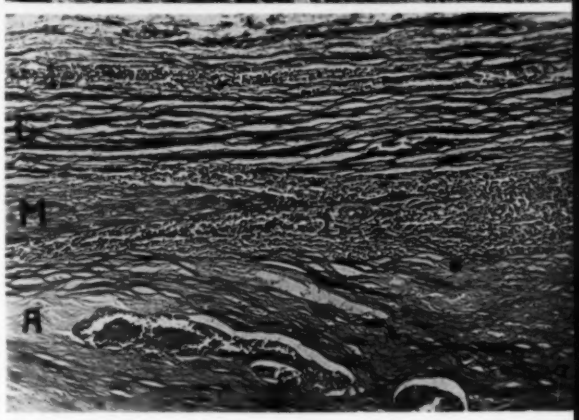

Fig. 3.—Section of the aneurysm wall showing focal dissolution of muscle, *M*, with a marked cellular inflammatory reaction and muscle fragments present in the defect. *C* is adventitial connective tissue and *A* is the expanding subintimal atheroma. Masson's trichrome stain; reduced ⅕ from mag. × 100.

Many of the arterial components of the vasa vasorum, and especially those adjacent or within these areas, contained varying degrees of sclerosis. There was a striking subintimal proliferation of fibrous tissue, with a consequent narrowing of the lumen (Fig. 5). In some cases this was marked, but in no case was it complete. A perivascular collection of lymphocytes was usually present, but no histologic evidence of arteritis or thrombosis was found.

Although no attempt is made to explain the etiology, the pathogenesis of the lesions

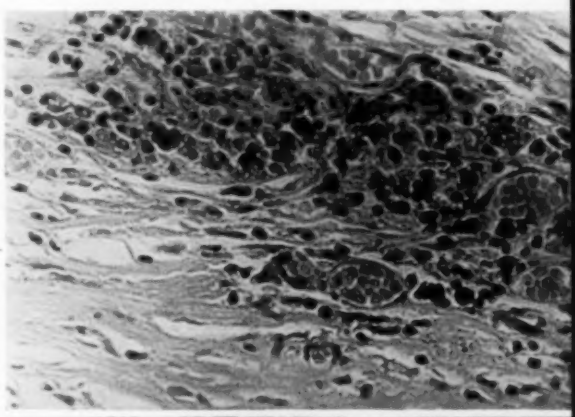

Fig. 4.—Higher magnification of a locus of muscle destruction. Lymphocytes, monocytes, and macrophages are present. Pigment is seen within the latter and lying free. The area is undergoing organization. Masson's trichrome stain; reduced ⅕ from mag. × 475.

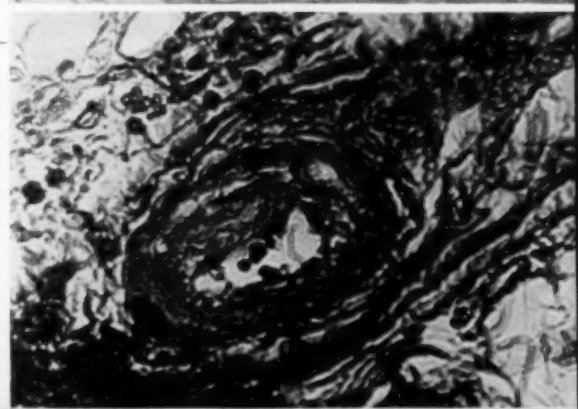

Fig. 5.—High magnification of an arteriolar component of the vasa vasorum. The lumen is markedly narrowed by the subintimal proliferation of connective tissue. This vessel was found adjacent to an area of muscle dissolution. Masson's trichrome stain; reduced ⅕ from mag. × 475.

in this particular case appeared to be twofold: (1) The more commonplace atherosclerotic changes of subintimal lipid deposits with fibrosis, endothelial ulceration, and thrombosis were accompanied by (2) a superimposed arteriosclerotic process caused by sclerosis of the arterial component of the vasa vasorum. As a result of the narrowed lumina and concomitant reduction in blood flow in the latter, the muscle of the media underwent progressive degeneration. This, in sequence, appeared to be cystic change, hemorrhage, cellular inflammatory reaction, complete dissolution, and fibrous replacement of muscle by connective tissue. The stronger containing power of the muscularis was lost. The relatively high intraluminal pressure then forced the expanding intimal plaque through the weaker fibrous tissue, and an aneurysm resulted. Histologic sections of the larger aneurysms frequently showed a thin layer of dense connective tissue about the periphery of the expanded intimal plaque, with strands of still recognizable muscle within the wall at varying distances to either side.

It is not known how frequently changes such as those described here in the vasa vasorum and media occur. A survey of the coronary arteries from 100 autopsies selected at random failed to produce any similar changes. The rarity of these particular findings may, in part, explain the rarity of aneurysms of the coronary arteries.

SUMMARY

A case of multiple aneurysms of both coronary arteries is described and a possible pathogenetic explanation suggested by the examination of the histologic sections is outlined.

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Lipogenesis by Cells of Cornea

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and
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In the course of a comprehensive study of fat metabolism in the cornea, we have observed a phenomenon which appears to be of sufficient interest to warrant a preliminary note. The observations indicate that fat may be formed in substantial quantities by all the native cells of the cornea (epithelium, endothelium, and stroma) and by certain other cells of the body, when supplied with proper substrates. The cornea is especially suited to studies on lipid histochemistry, since it is avascular, contains no stainable fat, and has a relatively simple cellular structure.

Although aberrant fat formation is a common finding in a variety of pathologic states, few direct studies have been aimed at determining the origin of the fat. It is generally assumed to result either from the simple liberation of masked fat in a degenerating tissue (lipophanerosis) or from blood lipids that are secondarily deposited in the necrotic zone. A large amount of literature based on indirect evidence has been advanced to support this latter contention. Yet it was pointed out in the early work on tissue cultures that individual cells were capable of forming fat from suitable substrates without necessarily any evidence of degeneration in the cells.* More recently, a most significant series of reports by Simms and collaborators has led to the conclusion that serum contains lipid-soluble substances, which they call lipfanogens, that can be taken up by chicken fibroblasts in tissue culture and converted into visible fat.† These substances are thought to play a role in atheroma formation. Various fatty acids are also found to be lipfanogens, but the authors doubt that they are of practical significance, for "none [of the fatty acids] has, by itself, enough activity to account for all the lipfanogenic activity of serum." The authors did not, however, study the effect of inactivating the fatty acid of serum; nor did they consider that an excess of the fatty acids may be present in those conditions associated with fat formation.

Our experiments support the concept of a potential lipogenesis by tissue cells other than the usual fat cells, provided suitable substrates, including serum, are present. In most respects they corroborate the contention of Simms and his co-workers but differ in indicating that both a serum factor and a fatty acid component are prerequisites in the media. In our corneal preparations neither of these constituents was alone capable of inducing appreciable lipogenesis, but together they were abundantly effective.

PROCEDURE

Corneal buttons, 3 to 4 mm. in diameter, were excised from rabbit eyes, injected with a small quantity of oleic acid‡ or 10% sodium oleate, incubated for varying lengths of time, and then prepared for histologic study. Most of the incubations were carried out in rabbit serum, 2 to 3 ml. of fluid per button. Frozen sections and paraffin sections were prepared. They were stained with Sudan IV,

Supported by funds from the American Heart Association and the Greater Boston Chapter of the Massachusetts Heart Association.

Howe Laboratory of Ophthalmology, Harvard Medical School, Massachusetts Eye and Ear Infirmary.

* References 1 and 2.

† References 3 through 6.

‡ Purified according to the method of Holman.[8]

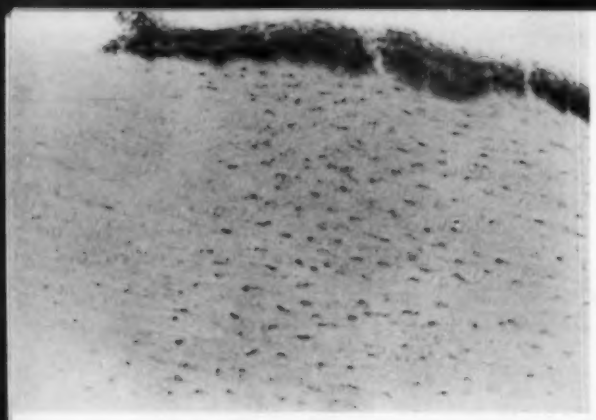
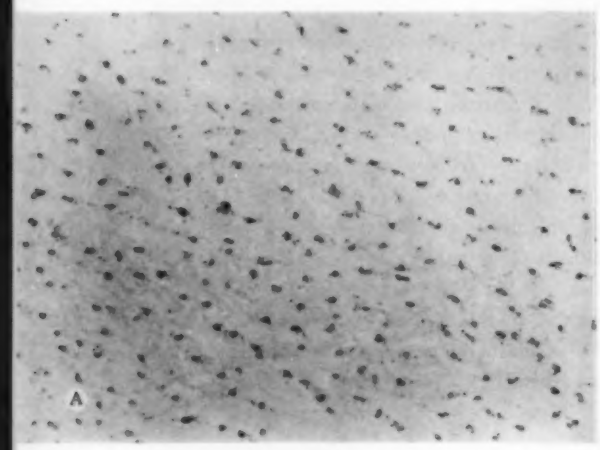
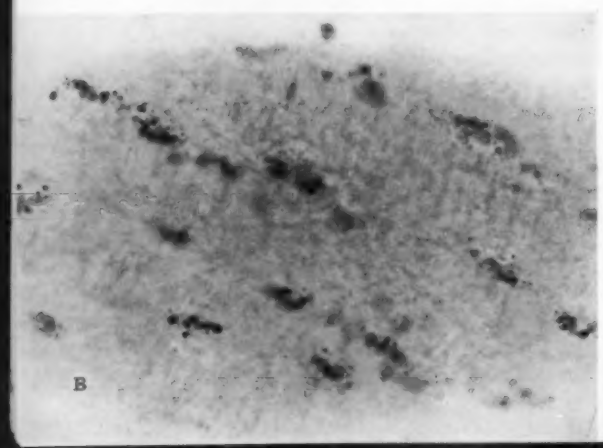


Fig. 1.—Anterior half of cornea adjacent to site of injection (oc. 4, obj. 16). Sodium oleate had been injected 24 hours previously in a region just to the left of the picture. Noteworthy are the acellular necrosis involving the stroma and epithelium, the adjacent area of epithelial and stromal cells containing sudanophilic globules, and the progressive decrease in sudanophilia away from the area of necrosis.

Fig. 2.—Sections of stroma from a corneal button which had been incubated with sodium oleate and serum for 24 hours (*A*, oc. 4, obj. 16; *B*, oc. 4, obj. 4). The specimen showed abundant sudanophilic globules in the cytoplasm of all the cells (including the epithelium, which is not shown in the photomicrograph).





Nile blue, hematoxylin and eosin, and according to the periodic acid-Schiff technique.§

This general technique was varied for specific purposes. The influence of pH was determined by adding buffering salts to the serum. Some of the buttons were exposed to 1% sodium fluoride or 1% potassium cyanide or heated to 60 C for one hour prior to the injection and incubation. In other experiments the serum was heated to 60 C for one hour prior to the incubation. The incubations were carried out under both aerobic and anaerobic atmospheres. The effect of several fatty acids was tested in addition to the oleate.

To avoid bacterial contamination, the experiments longer than 48 hours were carried out by placing the injected buttons within the anterior chamber or beneath the skin of another rabbit. This "incubation" in vivo provided not only longer observation periods but an opportunity to study the reactions of invading cells and of adjacent host tissue. The results in all these experiments were compared with those obtained by injecting oleic acid or sodium oleate directly into the intact cornea of living rabbits.

RESULTS

The observations may first be described for the buttons injected with oleic acid and incubated in untreated serum. After a few hours, the site of injection showed a mass of birefringent crystals. These took a dense blue stain with Nile blue and were occasionally faintly sudanophilic. They were interpreted as calcium soaps, since they frequently showed a positive von Kossa stain for calcium. In the corneal cells immediately about this mass the tinctorial affinities began to be lost in 6 to 12 hours and were practically gone in 24 hours (Fig. 1). Outside this necrotic zone fat globules began to appear after six hours, occurring simultaneously in epithelial, stromal, and endothelial cells. These globules then increased progressively in size for at least seven days and attained diameters of several micra (Fig 2). They

§ For the purposes of this paper, conventional interpretations of the lipid stains are accepted. Those substances which stain with Sudan are lipid solvents and, in the case of tissue, are usually noncrystalline fats. Those substances which stain pink with Nile blue are neutral fats, while those which stain blue include a large group of tissue components in addition to the injected fatty acids.

take a brilliant red with Sudan stains and a distinct pink with the Nile blue stain.‖

The fat was found only within the viable cells oriented about the site of injection. Thus, no fat was to be found in the degenerating cells in the zone of necrosis or in the cells at a considerable distance from the injected area. The most abundant fat was to be found in the surviving cells bordering on the site of necrosis.

Although showing the same qualitative changes as the stromal cells, the epithelium and endothelium appeared less susceptible to necrosis, and so occasionally a layer of fat-laden epithelium or endothelium remained bordering an acellular stroma.

Incubation of the epithelium or of the stroma alone with sodium oleate resulted in the same appearance of fat as when whole corneal buttons were incubated.

Variations in pH showed no fat in cells incubated in a pH range less than 4.95, slight fat at pH 5.6 to 5.7, considerable fat in the range 6.0 to 7.8, slight fat at pH 8.07 to 8.2, and none at pH 8.5 or higher. Fat was found in buttons incubated in an aerobic or nitrogen atmosphere, but none was found in buttons that had been immersed in 1% sodium fluoride or 1% potassium cyanide.

The appearance of fat was dependent on the presence of serum. No fat developed with incubations in isotonic sodium chloride solution with or without phosphate buffer (pH 7.8) or with the addition of glucose or glycerin. Neither did fat occur with buttons incubated in a moist chamber without serum. Horse serum could be substituted for rabbit serum. The essential factor in serum was not inactivated by heating to 60 C for one hour or by removal of the serum lipids by ether extraction.

Identical results were obtained with oleic acid, sodium oleate, or hydrolyzed rabbit fat. On the other hand, only a slight amount of fat occurred with injection of sodium stearate and none with injection of butyric acid or acetic acid, even though these were also necrotizing.

In the experiments involving implanting the button within the body, fat was observed in certain of the adjacent host cells. It was present abundantly in the invading fibrocytes and in the endothelium of the host cornea (when specimens were implanted into the anterior chambers). It was present to only a moderate degree in the macrophages and not at all in the polymorphonuclear cells. A similar differentiation of cells was noted with injections of oleic acid into the living rabbit.

In contrast to the foregoing observation on the appearance of fat, we have failed to find any evidence of lipolysis in the cornea. Neutral rabbit fat injected into the cornea of living rabbits induced no appreciable reaction for weeks, and then only a mild macrophage response, although injection of its hydrolytic products induced a violent inflammation. Neither have we been able to demonstrate any catalytic lipase activity by the Gomori [7] technique when corneal sections were incubated with stearate esters¶ and oleate esters.#

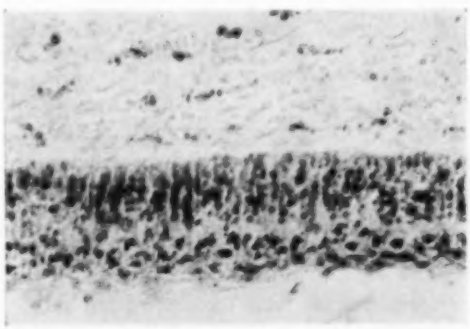


Fig. 3.—Anterior portion of a rabbit cornea incubated for 24 hours in serum and oleate media, stained with Sudan IV and hematoxylin. Noteworthy are the fat globules in the epithelial and stromal cells (oc. 10, obj. 4).

‖ Further evidence that oleic acid was not contributing directly to the sudanophilia is the uniform absence of Sudan "take" when buttons containing oleic acid or sodium oleate are incubated in aqueous media other than serum.

¶ "Tween 60" (Atlas Powder Co., Wilmington, Del.) and "Product 81" (Onyx Oil & Chemical Corp., Jersey City, N. J.).

"Tween 80" (Atlas Powder Co.).

CONCLUSIONS

The appearance of fat in these experiments is interpreted as being the result of a true lipogenesis dependent on the presence of certain viable cells, oleic acid or sodium oleate, and a serum factor. That it did not result from necrosis is evident from the facts that necrotizing agents other than oleates did not produce it; free fat is not necessarily in the necrotic tissue, and the fat appears only in the non-necrotic cells. Neither is it derived from blood lipids, since fat forms equally in the presence of defatted serum. Rather, it appears to be the formation of a neutral fat by an enzymatic process that is inactivated by sodium fluoride, potassium cyanide, or heat and has an optimum pH near neutrality.

The significance of these experiments lies in the fact that fat is shown to be formed abundantly by certain cells in the presence of substances that are native to the organism. It seems entirely possible that the appearance of fat in a variety of conditions, such as fatty degeneration, atheromatosis, and certain lipid histiocytoses, is not simply the unmasking of preformed fat (lipophanerosis) or the infiltration of the tissue by preformed lipids from the plasma but rather a dynamic process of fat synthesis in which oleic acid, derived exogenously or from a degenerating tissue, is the essential precursor. This potentiality of the corneal cells for lipogenesis is shared by fibrocytes, macrophages, and possibly many other cells in varying degree, but not by polymorphonuclear cells.

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Polyostotic Fibrous Dysplasia of Bone

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Polyostotic fibrous dysplasia of bone is a term suggested by Lichtenstein [1] in 1938 to identify the disease entity described in 1937 by McCune and Bruch [2] and Albright and his associates.[3] This entity, also called Albright's syndrome, is characterized by the presence of multiple cystic bone defects and, at times, abnormal cutaneous pigmentation and precocious sexual puberty in the female.

In 1942 Lichtenstein and Jaffe [4] reviewed the literature and recorded 90 cases, including 15 previously unreported ones in their own series of 23. They found that the fibro-osseous bone lesions constituted the nucleus of the disease. In only 32 of the 90 cases was there any abnormal cutaneous pigmentation. Only 20 of the 90 patients exhibited clinical manifestations of endocrine dysfunction. In their own series of 23 patients, only 2 showed abnormal skin pigmentation and 2 female patients gave history of significantly early menarche. Dockerty and his associates [5] in 1945 reported six new cases of fully developed polyostotic fibrous dysplasia or Albright's syndrome. They found 33 cases listed in the literature * between 1922 and 1942. All of these patients had extraskeletal findings.

No doubt, a great number of cases of polyostotic fibrous dysplasia with and without extraskeletal findings have been overlooked when reported under various titles. Lichtenstein and Jaffe [4] found 33 other titles under which relevant cases had been described. The majority of these cases were represented under such titles as "osteitis fibrosa," "osteodystrophia fibrosa," and "fibrocystic disease of bone" and were qualified by terms like "focal," "unilateral," "regional," and "disseminata."

Another group of interesting cases of polyostotic fibrous dysplasia have been reported as so-called osseous xanthomatosis or lipid granulomatosis of bone, without symptoms of the Hand-Schüller-Christian syndrome. Snapper and Parisel,[7] in 1933, reported such a case which Snapper † now accepts as one of polyostotic fibrous dysplasia, since the patient had multiple bone lesions, precocious puberty, and large nevi. They made the important differentiation of this entity from osteitis fibrosa cystica due to parathyroid adenoma. Keinboeck and Mewrach,‡ in 1932, described a patient whom they thought had generalized fibrocystic osteitis, but parathyroid exploration showed no tumor. A new rib lesion which

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The case presented was from the Surgical Service of Dr. Paul E. Walker, U. S. P. H. S. Hospital, Seattle, Wash.

Photomicrographs were taken by Mr. Jack Newby, of the University of Washington Photography Department. Photographs of the roentgenograms were taken by Mr. Donald Stuart, of the U. S. P. H. S. Hospital, Seattle. Chemical analysis of xanthomatous tissue was done by Mrs. Della Ramsden, Chief Technician of the Pathology Department of the University of Washington School of Medicine.

* Hummel, R., cited by Dockerty.[5]

† Snapper,[6] Chap. 12, pp. 209-224.

‡ Keinboeck, R., and Mewrach, L., cited by Snapper,[6] p. 209.

Fig. 1.—Roentgenogram showing fracture sites of the left femur and pseudocystic bone lesions of both left and right femur.

came to biopsy disclosed xanthoma or foam cells. Shelling and Voshell [8]; Snapper [9]; Layani, Ducroquet, and Loudat §, and Landoff [10] report other similar cases of so-called osseous xanthomatosis or lipid granulomatosis without signs and symptoms of the Hand-Schüller-Christian syndrome. In a case reported by Thannhauser and Magendantz [11] in 1938, xanthomatous pseudocystic bone lesions were found in their Case No. 20, a 4-year-old girl. This case may also fit into the category of xanthomatous polyostotic fibrous dysplasia.

Variability in the microscopic features of the disease is probably responsible for much of the confusion in classification. The most commonly reported microscopic feature of polyostotic fibrous dysplasia is that of fibro-osseous tissue with variable amounts of bone and fibrous tissue. Lichtenstein and Jaffe [4] state that occasionally foci of cartilage, giant cells, and foam cells may also be found. The presence of xanthoma or foam cells have been significant enough to warrant photomicrographs in reports by Windholz [12] and Dockerty and his associates.[5]

This following case report shows, as do those cases reviewed, that xanthomatous cells may be found as an important histological feature of polyostotic fibrous dysplasia.

§ Layani, F.; Ducroquet, R., and Loudat, M., cited by Snapper,[6] pp. 209-222.

REPORT OF A CASE

A 26-year-old seaman was admitted to the U. S. Public Health Service Hospital on June 4, 1951. He sustained a fracture of the left femur when he fell during a lifeboat drill.

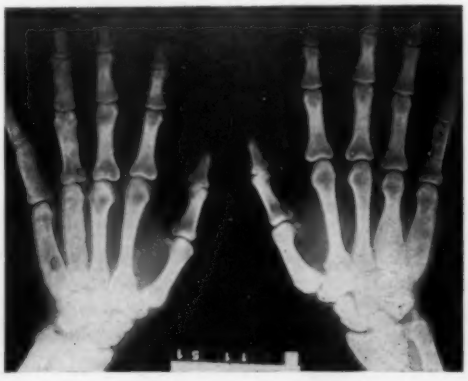
The patient gave a history of many fractures since early childhood, starting at the age of one year. He had fractured his left elbow twice, left femur three times, the fingers of both hands many times, his right arm and his right femur once.

On admission he complained of very little pain or discomfort. The temperature, pulse, respirations, and blood pressure were normal. There was no unusual pigmentation of the skin. Examinations of the head, neck, chest, and abdomen were not unusual. The left leg was 3.5 cm. shorter than the right, and there was some tenderness and lateral bowing of the upper portion of the left thigh.

Laboratory studies disclosed a negative serology, an erythrocyte count of 5,000,000, and a leucocyte count of 8100 cells per cubic millimeter. The hemoglobin was 15 gm. per 100 cc. and the sedimentation rate (Westergren) was 11 mm. The clear yellow acid urine with a specific gravity of 1.026 contained no albumin or sugar. Blood chemistry studies showed a serum calcium of 11.2 mg. per 100 cc., serum inorganic phosphorus of 4 mg. per 100 cc., acid phosphatase of 1.8, and alkaline phosphatase of 19.8 King-Armstrong units. Serum cholesterol was 176 mg. per 100 cc. The thymol turbidity, sulfobromophthalein (Bromsulphalein) excretion, and cephalin flocculation were normal.

Roentgenograms of the left femur showed pseudocystic rarefied areas of the upper third of the left femur and head, with recent fracture below the head and an older one through the neck. The neck was in contact with the ilium like a false head (Fig. 1). Skeletal survey roentgenograms also showed rarefied cystic-like areas of the metacarpals of both

Fig. 2.—Cystic areas of metacarpal of both hands.



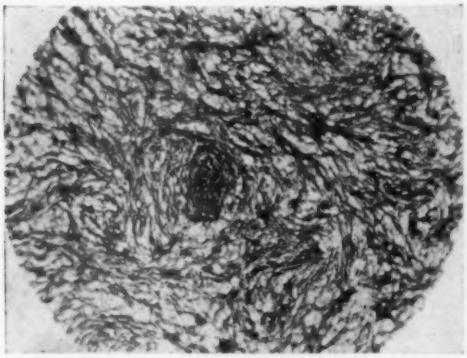


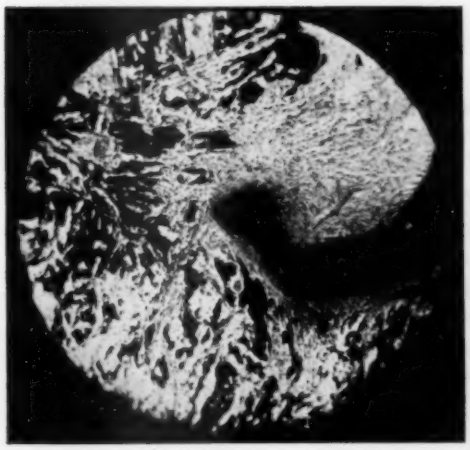
Fig. 3.—Photomicrograph of biopsy specimen from left femur. Foam cells with abundant cytoplasm and small nuclei are prominent. Hematoxylin and eosin stain; reduced about ½ from mag. × 80.

hands (Fig. 2), upper end of right femur (Fig. 1), left fibula, and left and right ulna. Skull roentgenograms were normal.

On June 5, an open reduction of the left femur was done and an Egger plate was inserted (Fig. 1).

Microscopically, a biopsy specimen consisted of dense masses of foam cells with a small amount of interspersed vascular collagenous tissue (Fig. 3) and a few scattered lymphocytes. Frozen sections and Sudan IV stain disclosed clusters of small needle-like crystals that were doubly refractile and formed crosses when heated. Crystals and

Fig. 4.—Frozen section of cystic bone lesion seen in both femurs. Black zones represent deposits of cholesterol esters, except for large bony spicule and fibrous tissue on the right. Sudan IV fat stain; reduced about ½ from mag. × 65.



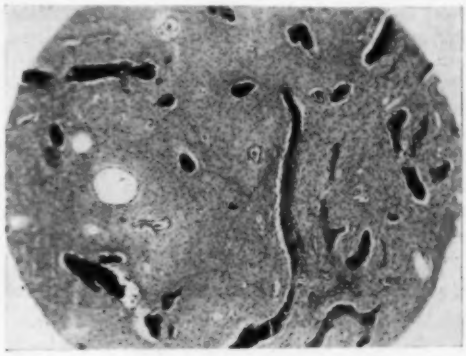


Fig. 5.—Photomicrograph of fibro-osseous tissue found in biopsy of left and right femur. Hematoxylin and eosin stain; reduced about ½ from mag. × 65.

fat globules were stained orange-red with the Sudan IV (Fig. 4). On July 23, biopsy of one of the rarefied areas of the other femur was done for comparison with the left. Two patterns of tissue were observed. Prominent masses of xanthoma cells identical with the first biopsy specimen were found (Fig. 3). The other tissue was of a fibro-osseous nature (Fig. 5), a classical example of fibrous dysplasia with irregular new bone trabeculae and a spindle fibrous stroma varying in compactness. No cartilage or prominent giant-cell groups were present.

On Oct. 9, a nonunion of the fracture was explored; another plate was inserted and on-lay bone grafts were applied. Postoperatively, a severe local infection with Pseudomonas and hemolytic Staphylococcus occurred. On Dec. 3, a sequestrectomy and

Fig. 6.—Postoperative roentgenogram. Cystic bone defects of the right femur are prominent.

plate removal were done, since pain and some drainage persisted from the fracture site. A third biopsy again showed xanthomatous tissue. X-ray therapy was given to both femurs to see if an improvement occurred. The pain and discomfort of the left leg continued, and no change occurred in the lesion. An amputation of the extremity was performed on Feb. 13, 1952 (Fig. 6). Owing to phantom leg pain a sympathectomy was later done. The patient was greatly improved and later fitted with a prosthesis.

The hemisected femur showed fibrous and fibrocystic replacement of the usual cancellous bone of the head and neck (Figs. 7 and 8).

There were also several prominent foci of soft yellow tissue, up to 1 cm. in diameter, adjacent to fibrous cysts. The cysts measured up to 3 cm. in diameter and they had a smooth glistening gray membranous lining.

Microscopically there were poorly cellular collagenous areas and areas of xanthomatous and fibro-osseous tissue. Frozen sections showed clusters of refractile needle-like crystals seen under the polarized light. Chemically the xanthoma tissue contained chiefly cholesterol esters, as listed in the following report:

Specimen.—Wet weight 100 mg.

Procedure.—Specimen macerated in 4 ml. saline and extracted in 100 ml. of hot acetone-alcohol mixture. Free cholesterol and cholesterol as esters determined by Sperry-Schoenheimer method. Lipid phosphorus determined by a modification of the Whitehorn and Fiske-Subbarow methods. Total lipid determined by evaporation of extract to dryness, extraction of residue with petroleum ether, evaporation of this extract to dryness, and weighing the residue.

Fig. 7.—Hemisected portion of resected femur showing multiple foci and fibrous areas.

Fig. 8.—Roentgenogram of hemisected specimen seen in Figure 7.

Results.—

	Mg. in 100 Mg. Specimen	Mg. per 100 Gm.
Lipid phosphorus	0.020	20
(Phospholipid as lecithin)	0.50	500
Free cholesterol	0.14	140
Esterified cholesterol	9.46	9,460
(If present as cholesterol palmitate)	15.30	15,300
Total lipid	14.2	14,200

COMMENT

The clinical, roentgenographic, and pathological features of this case place it in the category of polyostotic fibrous dysplasia. Due to the findings of abundant xanthomatous as well as fibro-osseous tissue in several biopsy specimens and in the amputated specimen, we have considered that xanthomatous tissue can be a prominent feature of polyostotic fibrous dysplasia, as was reported in other cases.‖

‖ References 7 through 12, and Layani, Ducroquet, and Loudat, cited by Snapper,[6] pp. 209-222.

GENERAL COMMENT

There is a general agreement among various authors that polyostotic fibrous dysplasia exists as a definite clinical entity.

The etiological basis is questionable and various suggestions are made as to the possible anomalous origin ¶ and hereditary,[13] endocrine,[14] neurogenic, and variable metabolic factors.[3]

On clinical grounds, the diagnosis of polyostotic fibrous dysplasia can easily be made if extraskeletal changes are present. As is well known, the majority of cases of polyostotic fibrous dysplasia show only the skeletal bone lesions and abnormal cutaneous pigmentation and precocious sexual developmental history are absent. Various other diseases also produce multiple pseudocystic bone lesions, and these include osteitis fibrosa cystica (parathyroid adenomas), Ollier's dyschondroplasia, hemangiomas, Letterer-Siwe disease, and osseous xanthomatosis of Hand-Schüller-Christian. The clinical history, physical examination, roentgenograms, and blood chemistry findings are needed before an accurate diagnosis can be made with a biopsy.

Grossly, the diseased bone has a grayish-white or yellowish tissue that replaces the normal cancellous bone and marrow. Occasionally cystic foci are found in the fibrous areas. Our case also showed soft foci of yellow xanthomatous tissue adjacent to fibrous cystic foci.

The histological picture of polyostotic fibrous dysplasia is variable and could be confused with Paget's disease,[11] osteitis fibrosa cystica,# giant-cell tumor [5] or so-called xanthoma and lipid granuloma of bone.* This variable histologic pattern is probably due to obtaining the biopsy specimen from lesions in various stages of development and, also, alteration may be due to recent fractures through the pseudocysts. Poorly cellular collagenous tissue may predominate and is the type found about the cystic portions. In more cellular areas, there are plump spindle cells and irregular spicules of bone. Beside the fibro-osseous tissue well demonstrated by Lichtenstein and Jaffe [4] xanthomatous tissue can predominate in some foci, as was demonstrated in this case. Snapper † believes that the xanthomatous tissue is the type found in early lesions of polyostotic fibrous dysplasia and that fibrosis develops in older lesions.

The growth pattern of polyostotic fibrous dysplasia would be better understood if serial biopsy specimens were made on cases followed from early childhood to maturity.

¶ References 1 and 4.

Keinboeck and Mewrach, cited by Snapper,[6] p. 209, and reference 7.

* References 7 through 11.

† Snapper,[6] Chap. 12, pp. 209-224.

SUMMARY

Cases of polyostotic fibrous dysplasia with prominent xanthomatous tissue are briefly reviewed.

A case of polyostotic fibrous dysplasia with prominent xanthomatous features is presented along with the chemical analysis of the tissue.

The presence of abundant xanthomatous or foam cells in a biopsy specimen of a cystic bone lesion should suggest the possible diagnosis of polyostotic fibrous dysplasia.

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News and Comment

ANNOUNCEMENTS

Congress of Comparative Pathology.—The Seventh International Congress of Comparative Pathology will be held at the Institut d'Anatomie Pathologique, Lausanne, Switzerland, May 26-31. The program will include papers and discussions on viral infections transmitted to man by animals, pathological and hygienic aspects of atmospheric pollution problems, and growth disturbances in comparative pathology. Official languages of the congress will be French, English, German, Italian, Spanish, and Russian. A technical exhibit will be provided, and visits to scientific institutions will be made. Postcongress tours from two to five days in Switzerland are planned. Titles and texts of communications must be submitted not later than May 1 to the General Secretary of the Seventh International Congress of Comparative Pathology, 19 rue César-Roux, Lausanne, Switzerland. Requests for the program and inquiries concerning registration should be addressed to the General Secretary, or to Lee M. Hutchins, Chairman, United States Section (Chief, Division of Forest Disease Research, Forest Service, U. S. Department of Agriculture, Washington 25, D. C.).

Examinations for Medical Officers.—A competitive examination for appointment of Medical Officers to the Regular Corps of the United States Public Health Service will be held in various places throughout the country on June 7, 8, and 9, 1955.

Appointments provide opportunities for career service in clinical medicine, research, and public health. They will be made in the ranks of Assistant and Senior Assistant, equivalent to Navy ranks of Lieutenant (j. g.) and Lieutenant, respectively.

Entrance pay for an Assistant Surgeon with dependents is $6,017 per annum; for Senior Assistant Surgeon with dependents, $6,918. Provisions are made for promotions at regular intervals.

Benefits include periodic pay increases, 30 days annual leave, sick leave, medical care, disability retirement pay, retirement pay which is three-fourths of annual basic pay at time of retirement, and other privileges.

Active duty as a Public Health Service officer fulfills the obligation of Selective Service.

Requirements for both ranks are U. S. citizenship, age of at least 21 years, and graduation from a recognized school of medicine. For the rank of Assistant Surgeon, at least 7 years of collegiate and professional training and appropriate experience are needed, and for Senior Assistant Surgeon, at least 10 years of collegiate and professional training and appropriate experience are needed.

Entrance examinations will include an oral interview, physical examination, and comprehensive objective examinations in the professional field.

Application forms may be obtained by writing to the Chief, Division of Personnel, Public Health Service, Department of Health, Education, and Welfare, Washington 25, D. C. Completed application forms must be received in the Division of Personnel no later than May 6, 1955.

Anomalous Connection of Pulmonary Veins with Normal Pulmonary Venous Drainage

Report of Case Associated with Pulmonary Venous Stenosis and Cor Triatriatum

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JAMES W. DuSHANE, M.D.
and
JESSE E. EDWARDS, M.D., Rochester, Minn.

In a discussion of the anatomic and functional disturbances of the pulmonary veins, it was pointed out in an earlier paper [1] that anomalous drainage of the venous supply of the lung need not be associated with a malformation of the pulmonary venous system. In certain instances of atrial septal defect the abnormal flow is almost entirely derived from the right lung. In these cases the functional effect may be almost identical with that which would result from anomalous termination of the pulmonary veins of the right lung into the right atrium or contributory vein thereof. Because of this circumstance, it seemed appropriate to classify anomalies of the pulmonary veins both from functional and from anatomic points of view. It was suggested that, when a pulmonary vein ends abnormally, the term "anomalous pulmonary venous connection" be used rather than the term "anomalous pulmonary venous drainage." [2]

It has thus been established that there may be anomalous venous drainage from a lung with normal venous connections, in some instances. In other instances, as portrayed by the case here reported, there may be anomalous connection of the pulmonary veins but normal termination of pulmonary venous blood flow. In this case, while there were in the pulmonary venous system striking anatomic deviations from normal, the route of the flow of blood from the lungs was normally into the left side of the heart. In this case there was, in addition, obstruction in the pulmonary venous pathway giving rise to the secondary phenomena of this condition. Also, in the region of the left atrium there was an accessory chamber, into which the pulmonary venous drainage was carried and from which the blood then flowed into the true left atrium. In the latter regard the heart may be said to represent an example of cor triatriatum.

Dr. Becu and Dr. Tauxe are Fellows in Pathology, Mayo Foundation.

From the Section on Pediatrics (Dr. DuShane) and the Section on Pathologic Anatomy (Dr. Edwards), of the Mayo Clinic and Mayo Foundation. The Mayo Foundation is a part of the Graduate School of the University of Minnesota.

REPORT OF CASE

Clinical Features.—A female infant, 5 months of age, was admitted to the Mayo Clinic with a history of normal birth and normal progress until the age of 6 weeks, when the appetite diminished and the rate of gain in weight slowed. At the age of 4 months generalized cyanosis, with crying, was noticed for the first time. Anemia was diagnosed on the basis of a blood-hemoglobin value of 59% of normal, and small quantities of blood were given by transfusion on three occasions. Temporary increase in strength and appetite occurred, but by the age of 5 months she was taking only 10 to 12 oz. of milk daily. Breathing was reported as having been rapid and somewhat labored during the preceding month.

Physical examination revealed a small, pale baby girl who was breathing rapidly with grunting expiration. The anterior fontanel was soft. The lungs seemed to be normal on auscultation. The pulse was rapid but the heart otherwise appeared to be normal. The liver was slightly enlarged, extending 3 cm. below the right costal margin. Pulsations of the

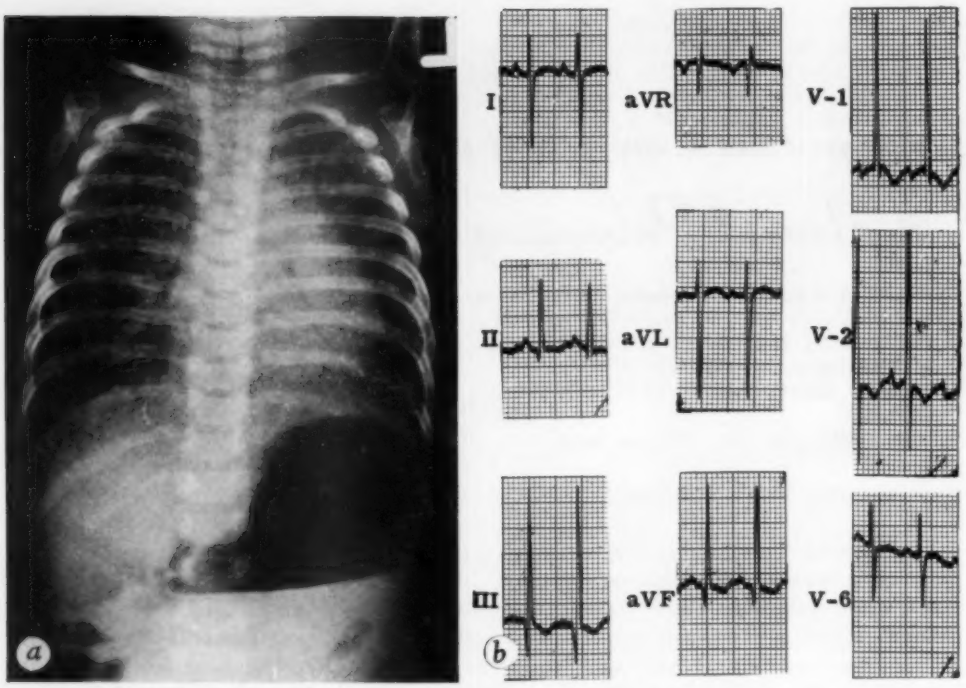


Fig. 1.—(*a*) Thoracic roentgenogram. Cardiac enlargement and increase of the pulmonary vascular pattern are demonstrated.

(*b*) Electrocardiogram. Right ventricular hypertrophy and probable right atrial enlargement are indicated. Note tall *R* waves in Lead V_1 and exaggerated *P* waves in Lead II.

femoral artery were normal. There was slight limitation of abduction of the hips.

The baby was admitted to the hospital at midnight and died suddenly 6 hours later.

Review of the roentgenograms of the thorax, taken by the referring physician, revealed cardiac enlargement and considerable increase in the pulmonary vascular markings (Fig. 1*a*). The electrocardiogram was interpreted as demonstrating right ventricular hypertrophy and probable right atrial enlargement (Fig. 1*b*).

A clinical diagnosis of congenital heart disease of undetermined type and possible congenital dysplasia of the hip was made.

Pathologic Features.—Of chief interest in the necropsy were the nature and connections of the pulmonary veins and the condition of the heart (Fig. 2). The left atrium appeared in two portions. One was the larger. A smaller accessory chamber lay above the larger portion. The lower and larger portion of the left atrium communicated with the auricular appendage, with a normal mitral valve, and with the tract of a valvular competent patent foramen ovale. The over-all measurements of the larger portion of the left atrium were about 2 cm. from side to side and 1.5 cm. in height. The larger portion had in its superior wall an aperture, which measured 1.1 cm. in diameter, through which the two portions of the left atrium communicated. The smaller (accessory) portion of the left atrium measured 1 cm. from side to side, 5 mm. from above downward, and about 5 mm. from before backward.

Two pulmonary veins, the left lower and the right lower, joined the respective lateral extremities of the accessory portion of the left atrial chamber (Figs. 3 and 4). The left lower pulmonary vein was narrow over a long distance, measuring about 1 mm. in its outer dimension along its entire course. The orifice of the left lower pulmonary vein was so narrow or so nearly occluded that it would not receive a probe measuring 0.5 mm. in diameter. The external diameter of the right lower pulmonary vein was comparatively wide, measuring 6 mm. At a point 0.5 cm. distal to the junction of this vein with the accessory portion of the left atrium, the lumen of the vein was stenosed by a circumferential focus of intimal gray tissue, being narrowed here to a diameter of only 3 mm. Histologically this focus consisted of relatively avascular connective tissue containing no elastic tissue but much collagen.

Inferior to the tracheal bifurcation and superior to the accessory left atrial chamber was a horizon-

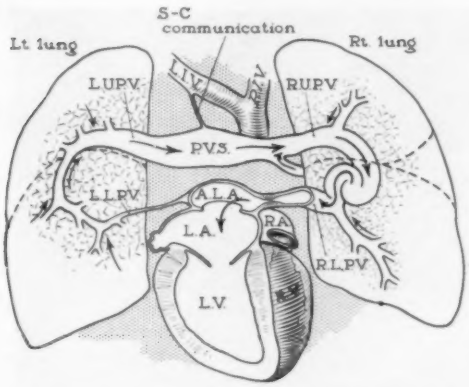


Fig. 2.—The pulmonary venous system and the left side of the heart, viewed from behind. The left lower pulmonary vein (*L. L. P. V.*) is a narrow channel. Blood from the lower lobe of the left lung flows into the left upper pulmonary vein (*L. U. P. V.*) and thence into the pulmonary venous sinus (*P. V. S.*). This sinus also receives blood from the middle lobe of the right lung. The blood in the pulmonary venous sinus is carried by way of anomalous connections with the right lower pulmonary vein into an accessory left atrial chamber (*A. L. A.*). From this chamber blood flows through a relatively wide opening into the left atrium (*L. A.*). In the right lower pulmonary vein (*R. L. P. V.*) there is localized stenosis. Between the pulmonary venous sinus (*P. V. S.*) and the left innominate vein (*L. I. V.*) there is a narrow connection (*S-C communication*) representing an embryonic connection between the splanchnic plexus and the cardinal system of the veins of the embryo. This communication, although narrow and unimportant functionally, is interesting developmentally. Photographs of the specimen from which this illustration was made are shown in Figures 3 and 4.

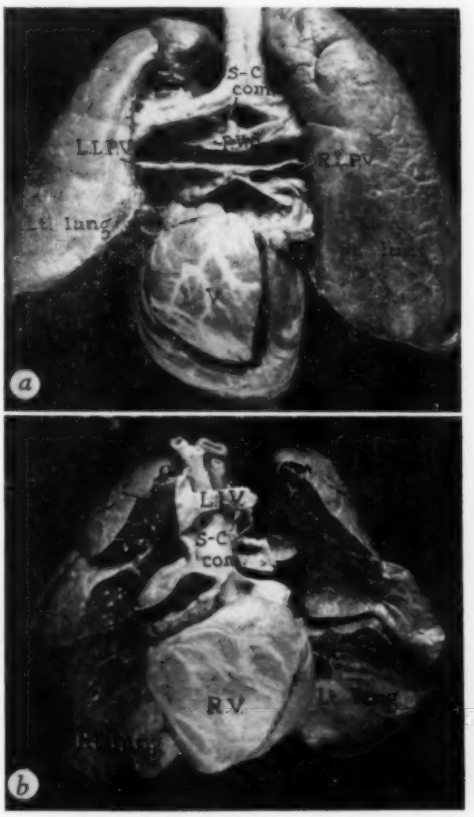
tally lying venous sinus, measuring 6 mm. from above downward and extending from the hilus of the left lung to the hilus of the right lung. At its left extremity this sinus received a vein, emanating from the upper lobe of the left lung. Along its right aspect the sinus received veins from the upper and middle lobes of the right lung and from the upper portion of the lower lobe of the right lung. This sinus did not communicate directly with the heart. At about the midline and from its upper extremity the sinus gave off a narrow branch, measuring 1 mm. in diameter, which ran vertically and upward in front of the proximal portion of the left main bronchus. As this branch ascended, it lay lateral to the ligamentum arteriosum and in front of the aortic arch. It then joined the inferior aspect of the left innominate vein at the origin of the latter. This branch did not admit a probe 0.5 mm. in diameter.

Histologically this branch was shown to have a very narrow lumen in which was vascular connective tissue. In its wall was identifiable a layer of elastic tissue, peripheral to which was a thick layer of dense collagen, interpreted as adventitia (Fig. 5*a*). Central to the elastic layer was a mixture of muscle and fibrous tissue, interpreted as representing intima and media of the vein. The left innominate vein joined the right innominate vein in a normal manner to form the superior vena cava.

The pulmonary veins were followed by dissection into the hilar region of both lungs (Fig. 4). The peripheral portion of the left lower pulmonary vein received tributaries from veins of the lower lobe of the left lung and, increasing in diameter, it arched upward and forward to join the left upper

Fig. 3.—(*a*) Posterior aspect of heart and lungs. Illustrated are the narrow left lower pulmonary vein (*L. L. P. V.*), the wider right lower pulmonary vein (*R. L. P. V.*), the pulmonary venous sinus (*P. V. S.*), and the lower portion of the connection between the pulmonary venous sinus and the left innominate vein (*S-C com.*). For details see Figure 4.

(*b*) Anterior view of heart and great vessels. The upper portion of the connection (*S-C com.*) between the pulmonary venous sinus and the left innominate vein is shown. The ventricular portion of the heart is represented anteriorly by the hypertrophied right ventricle.



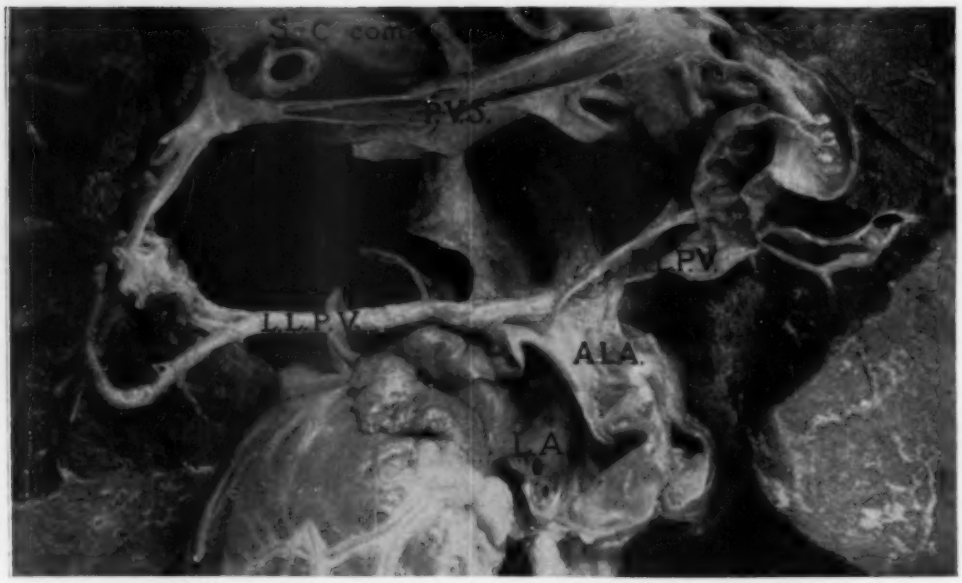


Fig. 4.—Details of pulmonary veins viewed from behind. Abbreviations are as used in Figures 2 and 3. The substance of the hilar regions of both lungs has been dissected away to reveal the dilated tortuous connection between the peripheral portion of the pulmonary venous sinus and the right upper pulmonary vein, on the one hand, and the right lower pulmonary vein, on the other. In the latter vein, close to its connection with the accessory left atrial chamber there is granularity of the lining, representing the thickening of the intima which narrowed the lumen and which is portrayed microscopically in (*b*), of Figure 5.

pulmonary vein which emanated from the upper lobe of the left lung. Therefore, the left upper pulmonary vein carried the blood of the whole of the left lung into the horizontally lying pulmonary venous sinus already described. A greatly dilated and tortuous vessel, measuring approximately 8 mm. in diameter, connected the right extremity of the pulmonary venous sinus with the right lower pulmonary vein.

In Figure 2 are illustrated the pulmonary venous pathways in this case. The narrow states of the proximal portion of the left lower pulmonary vein and of the connection between the pulmonary venous sinus and the left innominate vein seem to have precluded any major flow through these channels.

Venous flow from all of the left lung and the middle lobe of the right lung was carried into the pulmonary venous sinus. From this collecting sinus and from the right upper pulmonary vein the blood was carried by way of an anomalous connection between the right upper and lower pulmonary veins to the accessory left atrial chamber. The blood from the lower lobe of the right lung flowed directly to the accessory left atrial chamber by way of the right lower pulmonary vein.

Two sites of obstruction to the drainage of blood along the pathways just described were identified. The first, a site of severe obstruction, was at the level of the focus of intimal fibrous thickening described in the right lower pulmonary vein proximal to its junction with the accessory left atrial chamber (Fig. 5*b*). The second, a site of mild stenosis, was at the point where the accessory left atrial chamber communicated with the left atrium.

There was marked hypertrophy of the right ventricular wall associated with some dilatation of this chamber. This wall measured about 1 cm. in thickness and formed the major portion of the anterior surface of the heart (Fig. 3*b*). The left ventricle, which was of normal size, appeared by comparison as an appendage of the right ventricle. The whole of the left margin of the heart was formed by the right ventricular wall; the left ventricle lay posteriorly. The pulmonary trunk was dilated. The aorta and its branches were normal and the ductus arteriosus was closed. The ventricular septum was intact. A valvular competent patent foramen ovale was present.

Grossly the lungs were edematous. In addition, prominent and dilated lymphatic channels were noticed in the visceral pleura of both lungs.

Sections prepared from all regions of the lungs presented essentially similar histologic pictures. There was extreme capillary engorgement. Intra-alveolar hemorrhage and edema were prominent. There was hypertrophy of the media of the large

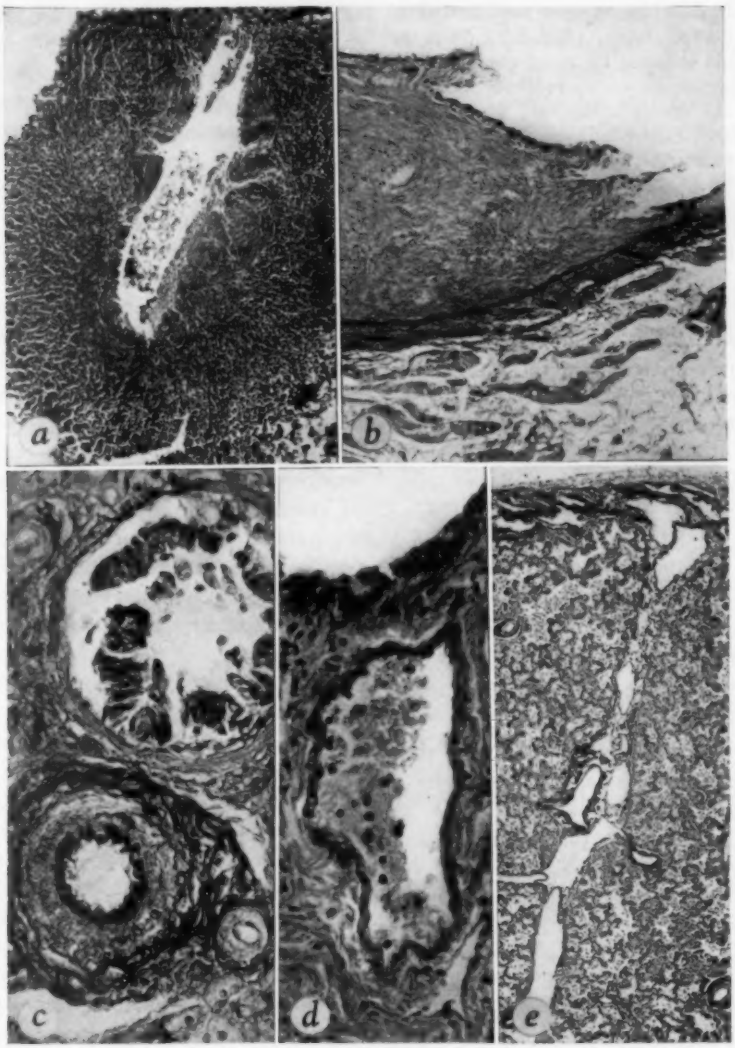


Fig. 5.—(*a*) Cross section of the connection between the pulmonary venous sinus and the left innominate vein illustrated in Figures 2, 3, and 4. Considerable collagenous tissue is in the adventitial portion of this narrow venous trunk. The intrinsically narrow lumen contains vascular connective tissue, resembling organized thrombus, which further narrows the lumen. Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 60.

(*b*) Longitudinal section through the right lower pulmonary vein at the site of localized narrowing. The narrowing is caused by intimal thickening, with relatively acellular connective tissue. Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 95.

(*c*) A large and a small muscular artery of the lung from the case presented, showing medial hypertrophy of both vessels. The internal elastic lamina is particularly prominent in the larger artery. Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 250.

(*d*) Control section showing a normal large muscular artery from a female patient aged 4½ months. Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 250.

(*e*) Section of the pleura and lung from the case presented, showing dilated lymphatics in the visceral pleura and in an interlobular septum. Congestion and edema also are present. Verhoeff's elastic tissue stain counterstained with Van Gieson's connective tissue stain; × 27.

and the small muscular arteries associated with thick internal elastic layers (Fig. 5*c*). That the medial thickness exceeded the range of normal was shown by comparison of micromeasurements of small pulmonary vessels in this case with those of controls (Fig. 5*d*). Lymphatic dilatation was prominent in the pleura and interlobular septa of both lungs (Fig. 5*e*).

COMMENT

The anatomic pattern of the pulmonary venous system in the case reported is complicated and, superficially, may seem difficult to explain. It is, in fact, easily understood when compared with the normal development of the pulmonary venous system. In consideration of the development of the pulmonary veins, it is essential to recognize that the lungs represent a localized elaboration of the primitive alimentary canal and in this regard the lungs, in their early stages, share their blood supply with the foregut.[3] In this way the capillary plexus of the lungs, which may be called the splanchnic plexus, has venous connections both with the cardinal system of veins and with the umbilicovitelline system of veins.

By these connections the primitive pulmonary venous system has potential connection with systemic veins whose origin is in the cardinal system, on the one hand, and with abdominal veins such as the portal vein and its tributaries and sinus venosus, on the other hand. These primitive connections disappear after the pulmonary venous system makes a connection with an outpouching of the atrial portion of the heart.[4] This process establishes the basis for the adult condition wherein there is direct connection between the pulmonary veins and the left atrium. In the case which we have presented, the connecting narrow channel between the common pulmonary venous sinus and the left innominate vein may be interpreted as representing persistence of an embryonic connection between the splanchnic plexus and the cardinal system of veins. The channel, on gross examination, was exceedingly narrow. Microscopically the intrinsically narrow channel was almost closed by vascular connective tissue, possibly representing an organized thrombus. It was not an effective venous channel. It is interesting to recall that in this vein the heavy deposition by collagenous tissue peripheral to the muscular layer (Fig. 5*a*) resembles a phenomenon seen in the umbilical vein undergoing postnatal obliteration of its lumen.

The common pulmonary venous sinus by which the two lungs communicated is considered a derivative of the splanchnic plexus, as it had been related to the lung buds. Initially the anlage of the lungs is one protrusion from the ventral wall of the foregut. Later this protrusion becomes elaborated into two branches, representing the anlage of each lung. As the branching of the lung bud occurs, the splanchnic plexus is carried to either side of the midline. That portion of the splanchnic plexus in the region of the midline which connects the vascular system of both the right and left developing lungs may be viewed as the tissue making up the pulmonary venous sinus observed in our case.

The morphologic state of the left lower pulmonary vein is similar to that described for the narrow connection between the pulmonary venous sinus and the left innominate vein.

The localized stenosis of the right lower pulmonary vein was formed by cellular nonvascular fibrous tissue (Fig. 5*b*). The basis for the development of this tissue is not clear, but the narrow state of the vein, caused by it, would seem to have been responsible for obstruction to pulmonary venous outflow from both lungs. The presence of pulmonary congestion and edema may be understood, if it is thought of as a result of the obstruction to pulmonary venous flow. Hypertrophy of muscular arteries, both large and small, and the associated presence of coarse internal elastic layers are consistent with changes seen in other forms of pulmonary venous obstruction, as in acquired mitral stenosis.

The prominent lymphatics involving the pleura and interlobular septum of the lungs represent an interesting phenomenon. We have observed it in other forms of congenital cardiac disease associated with obstruction to pulmonary venous flow. The hypertrophy

of the right ventricular wall, which was present in the case presented, may be viewed as an example of hypertrophy resulting from or associated with pulmonary hypertension initiated by the pulmonary venous obstruction.

The narrow left lower pulmonary vein calls to mind the case, reported by our group earlier, in which all of the pulmonary venous system joined to form a common sinus from which blood flowed to the superior vena cava by way of an anomalous connection. In addition, in that case there was an atretic strand of tissue which extended from the lower aspect of the common pulmonary venous sinus to the left atrium. We were of the opinion that this structure represented the common pulmonary vein of the embryo, which either remained narrow or had become narrow.[5] Whichever explanation is correct, the process involving the left lower pulmonary vein in the present case may have been similar to that responsible for the atretic strand in the other case mentioned.

With regard to the left lung in the present case, its venous drainage was through abnormal channels into the left atrium. It is an interesting circumstance that in this case anomalous pulmonary venous connection was associated with normal drainage. In earlier publications we recommended that "anomalous pulmonary venous connection" be used as an anatomic term, while "anomalous pulmonary venous drainage" be used as a functional term. At that time we emphasized the feature that in atrial septal defect there might be anomalous drainage from one lung with normal pulmonary venous connections. In our present case we have a reverse situation, in which anomalous connection of pulmonary veins was associated with normal termination of pulmonary venous flow into the left atrium.

The feature of the left atrium represented by a smaller and accessory chamber that received the pulmonary veins and a larger chamber that communicated with the auricular appendage and the mitral valve is characteristic of the condition called "cor triatriatum." It is controversial as to whether this condition represents a developmental anomaly of the pulmonary venous system or a malformation of the cardiac septa.[6] Others and we have suggested that it represents the former, namely, failure of incorporation of the common pulmonary vein of the embryo into the left atrium of the heart.[5] In this line of reasoning the accessory chamber is interpreted as representing the common pulmonary vein and the larger left atrial chamber is interpreted as representing the true left atrium of the embryo. The fact that, in the present case, cor triatriatum was associated with obvious developmental abnormalities of the pulmonary veins is circumstantial evidence that the element of cor triatriatum is also a manifestation of pulmonary venous malformation.

In some cases the opening between the accessory chamber and the true left atrium is so small as to constitute an important point of obstruction to pulmonary venous flow. Such cases are exemplified by those of Church,[7] reported in 1868; Borst,[8] reported in 1905, and others, presented more recently.* In our present case this opening was relatively wide, resembling in that regard the situation in the cases of Griffith,[10] reported in 1903, and Loeffler,[9] reported in 1949. In our case the wide opening between the accessory left atrial chamber and the true left atrium cannot be considered to have exerted an important impediment to pulmonary venous flow. As has been mentioned, however, there was significant obstruction to the pulmonary venous flow created by the circumstances of the anomalous pulmonary venous connections and the stenosis of the right lower pulmonary vein. The obstruction to pulmonary venous flow at this site is interpreted as being responsible for elevated pulmonary venous, capillary, arterial, and right ventricular pressures. It is considered to have caused death from pulmonary edema. The condition, in this case, is one to be added to those to be entertained in the differential diagnosis of mitral stenosis.[14]

* References 5 and 9 through 12.

SUMMARY

This is a report of the case of a 5-month-old female infant with anomalous connection of the pulmonary veins but with normal flow of pulmonary venous blood to the left atrium. In addition, stenosis of the pulmonary venous system of severe degree existed. This was considered responsible for pulmonary edema and evidences of pulmonary hypertension. Also present was a condition, cor triatriatum, which did not seem to contribute to the functional disturbance. From the developmental point of view the latter condition is thought to be related to malformations of the pulmonary veins.

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Radioautographic Localization of Na^{22} in the Rat Kidney

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Rats, under various experimental conditions, were injected with radiosodium (Na^{22}) and the patterns obtained by exposure of frozen-dried kidney slices to photographic film were then interpreted on the basis of the several factors involved. Through use of this general technique it has been possible to visualize ions in various regions of the rat kidney and to correlate these findings with theories of renal function.

The problem of renal function in relation to electrolytes has been approached in two principal ways, by micromanipulation of kidney preparations with analysis of the various fluids obtained and by indirect observations using renal function tests. Application of these methods and correlation of the resulting data have led to theories that attempt to explain both function and sites of free ion activity within the kidney.

Although many data have been accumulated, there are still large gaps in our knowledge of renal electrolyte metabolism. A major cause is that most of the important ions, such as sodium, potassium, calcium, chloride, and phosphate, are so widely distributed that the best results have come by means of indirect observations. It is well recognized, however, as Smith [1] points out, that interpretations based on such determinations "sometimes lead to semasiologic difficulties." This paper describes an attempt to use radioautography as a direct means of studying renal mechanisms in the metabolism of electrolytes.

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MATERIALS AND METHODS

Sprague-Dawley albino rats weighing approximately 200 gm. were used throughout. The animals were kept in air-conditioned rooms at 21 C and were housed in glass metabolism cages during chronic experiments. Two diets were used: commercial Rockland rat diet (control diet) and a low-sodium diet (Table 1). The control diet had a sodium content of 38 mEq/100 gm. The sodium content of the special diet was 0.42 mEq/100 gm.

The isotope used was Na^{22} in the form of the chloride.* It was chosen in preference to Na^{24} because the 14.8-hour half-life of the latter would have required the use of potentially toxic doses. The radiosodium was injected intravenously or intraperitoneally in 5μc or 10μc quantities in 1 or 2 cc. of 0.9% sodium chloride, respectively. Intravenous injections were always given into the inferior vena cava of ether-anesthetized animals. Intraperitoneal injections were made without anesthesia. All animals were killed by a blow to the head or by an overdose of anesthetic administered at the end of the experimental period.

Serum and urine sodium and potassium determinations were made, using a Beckman DU spectrophotometer with flame attachment. Estimations of radioactivity in the injected samples, serum and urine, were performed in the usual fashion with a Geiger-Müller counter, and corrections were made for self-absorption in the samples.

* Dr. Philip H. Abelson, of the Carnegie Institution of Washington, Institute of Terrestrial Magnetism, supplied the Na^{22} used in this study.

Histologic and Radioautographic Technique.—Following the appropriate experimental procedure, the kidneys were removed as rapidly as possible and frozen in isopentane at liquid nitrogen temperatures (—160 C to —190 C). The tissues were dehydrated while frozen,† embedded in paraffin, and sections, 6μ to 25μ thick, were cut by the paraffin backing technique. The details of this procedure are described by Jennings.‡

Radioautographs of these sections were then prepared. Small squares of photoroentgen film § were closely applied to the section, emulsion side up. All sections were stored in a desiccator at 4 C to 10 C throughout the exposure period. At empirically determined intervals, the films were developed as recommended by the manufacturer. The best definition was obtained by underdeveloping overexposed films, as suggested by Evans.[4] These autographs were then compared with the same sections after staining. Toluidine blue was usually used as a stain for the sections because fewer manipulations are required, thus circumventing major distortions of the tissue during staining. Sections mounted directly on Autoradiographic A or No Screen film were stained with metanil yellow Harris hematoxylin, as described by Simmel, Fitzgerald, and Godwin.[5] The use of metanil yellow eliminates deep staining of the gelatin emulsion, which usually occurs when most other stains are used, and thus allows good visualization of the section and autograph. Formalin-fixed hematoxylin and eosin stained sections of kidney, liver, adrenal, heart, and lung were studied routinely.

TABLE 1.—*Composition of Diets*

Composition of Low-Sodium Diet	Gm.
Vitamin-free casein	18.0
Brewers' yeast, dry	5.0
Sucrose	66.7
Crisco (R)	5.0
Salt mixture:	
Calcium carbonate	2.0
Potassium chloride	1.0
Magnesium oxide	0.2
Potassium acid phosphate	1.5
Ferrous citrate	0.5
Cupric sulfate, cryst	0.1

Composition of Control Diet (Rockland rat diet, complete, in pellet form)	Per Cent
Protein	21.00
Fat	4.00
Fiber	6.00
Carbohydrate (mixed) approx	59.00
Silica (SiO_2)	0.59
Chlorine (Cl)	0.96
Sodium (Na)	0.88
Potassium (K)	1.12
Calcium (Ca)	2.13
Phosphorus (P)	0.71
Sulfur (S)	0.24
Magnesium (Mg)	0.33
Iron (Fe)	0.00037
Plus trace elements and vitamins	

Experiments.—Three general types of experiments were performed. In all experiments, autographs were made by the method described above.

The first group of experiments were carried out in an attempt to define the vascular pattern of the rat kidney as it appears on a radioautograph. Two

† The kidneys are dehydrated while frozen in order to prevent any movement of sodium from its location at the time the kidney was frozen. The theoretical minimum dehydration temperature necessary to prevent such a shift of sodium is the eutectic point of aqueous sodium chloride, —21.2 C. Measured dehydration temperatures of —30 C to —40 C were used throughout these experiments. Conceivably shift or diffusion of ions could occur at the dehydrating temperatures used. However, we believe this did not occur because of the following observations: (*a*) Variations in dehydration temperature from —50 C to —23 C gave identical radioautographs after exposure of the sections. (*b*) No shift of localization was observed when autographs from tissue containing marked ice crystal artifact were compared with autographs from properly frozen control kidneys. (*c*) Experiments in progress on other diffusible ions and molecules using the same technique also show definitive localization. For example, Ca^{45} and iodinated I^{131} human serum albumin in similarly prepared kidneys can be localized in specific segments of the cortex. Had there been postmortem diffusion, the resultant exposure would have been diffuse rather than localized.

In essence, this dehydration is a slow precipitation. It is conceivable that, as such a process goes on, there might be a shift of ions from one portion of the tissue to another. We have not been able to demonstrate any such shift, either among the various zones or from one level of a section to another as determined by serial sectioning. It is true that, in vitro, frozen solutions will show local concentration of electrolytes following thawing, an observation previously made by each of us independently. However, this is not the case when solutions are frozen as quickly and completely as in these experiments. Beyond certain limits, the key to effective avoidance of shifts lies in the speed of freezing more than in the temperature of dehydration.

‡ References 2 and 3.

§ This is a medium contrast, high-speed film. It is inexpensive, readily available, and provides adequate definition for these purposes. For good resolution, Eastman Autoradiographic A and No Screen Film were used.

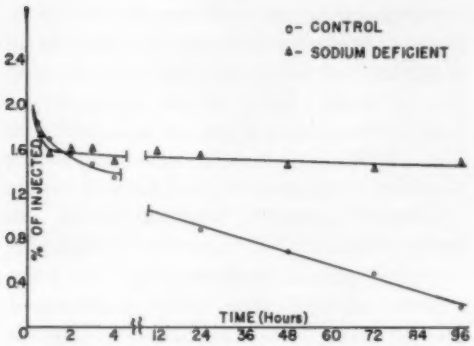


Fig. 1.—Per cent of injected dose of Na^{22} per milliliter of plasma at intervals following intraperitoneal injection.

methods were used. In the first procedure, 1.5 ml. of autogenous P^{32}-labeled erythrocytes, which had been labeled by the technique of Reid and Orr,[6] were injected intravenously in saline solution into the inferior vena cava of the anesthetized rat. The renal pedicles were then clamped at intervals of 0.5, 1, 3, and 4 minutes after the start of the injection. In the second methods, Na^{22} was used for the same purpose. The approach in this case was to clamp renal pedicles in anesthetized animals at intervals from 15 seconds to five minutes after the start of the intravenous injections of 5μc of Na^{22}. Two groups of animals were used in this series: one group on the normal diet and one on the low-sodium diet.

The next group of experiments consisted of a series in which the autoradiographic pattern of kidneys from normal animals was obtained and compared with similar observations of sodium-deficient rats. In this group, the isotope was injected intraperitoneally and the animals were killed at intervals of from 30 minutes to seven days following the injection.

In the third group of experiments, the changes induced by pharmacologic agents thought to affect sodium metabolism in the kidney—organic mercurials, acetazoleamide (6063; Diamox),‖ vasopressin injection (Pitressin), corticotropin, cortisone, and desoxycorticosterone (Doca) acetate—were investigated. The data on the management of each of these are included under results.

RESULTS AND OBSERVATIONS

Average sodium levels in our animals as determined on the flame spectrophotometer were: controls, 152 mEq/liter; low-sodium group, 142 mEq/liter of plasma.

‖ Dr. R. O. Roblin Jr., of the American Cyanamid Company, supplied the acetazoleamide (6063) used in these experiments.

Figures 1 and 2 represent graphically the average changes in plasma levels and urinary excretion of Na^{22} as obtained in the various experiments. The plasma levels were obtained from rats injected intraperitoneally. Data on plasma following intravenous injection are available in the literature [7] and show that the equilibrium level is attained in from two to seven minutes. We could not perform these counts on our experimental animals for fear of compromising the conditions of the experiments. Our results show that an equilibrium period is reached within 30 minutes following intraperitoneal injection and is maintained for approximately four hours in normal as well as sodium-deficient animals (Fig. 1). In studies extending up to four days following injection, it is seen that the percentage of injected counts falls in the normal animals and remains at a fairly high level in the sodium-deficient group. This finding is essentially a corollary of that found in the studies on urinary excretion. The plasma curves are independent of the initial dose, since parallel curves were obtained when injections of both 5 μc in 1 ml. of 0.9% NaCl and 10μc in 2 ml. of NaCl doses were compared.

The data on urinary excretion plotted in Figure 2 are based on 24-hour analyses and are almost identical with those of Greenberg, Campbell, and Murayama.[8] It is noteworthy that in both groups the rate of excretion of

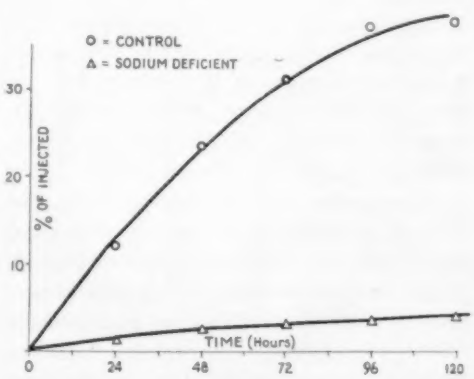


Fig. 2.—Per cent of injected dose of Na^{22} recovered from the urine of rats on the low-sodium and control diets.

TABLE 2.—*Typical Twenty-Four Hour Urinary Excretion Data*

Day	Na^+ — mEq/ 24 Hr.	K^+ — mEq/ 24 Hr.
No. 56—Control diet		
1	5.16	1.63
2	4.50	2.20
3	4.72	2.10
4	4.80	1.47
5	4.10	1.91
6	2.85	....
7	2.16	1.20
2 ml. 0.9% NaCl intraperitoneally		
8	2.84	1.66
9	4.75	3.61
10	3.17	2.18
No. 51—Control diet		
1	3.74	1.50
2	5.20	1.83
3	4.32	2.00
4	3.43	1.69
5	4.11	2.37
6	4.13	1.44
7	3.87	....
Diet changed to low sodium		
8	....	....
9	0.88	....
10	0.018	....
11, 12, 13, 14	0.017	....
15	0.005	0.90
16	0.006	2.18
17	0.010	3.16
2 ml. 0.9% NaCl intraperitoneally		
18	0.224	1.52
19	0.034	1.71
20	0.023	1.65

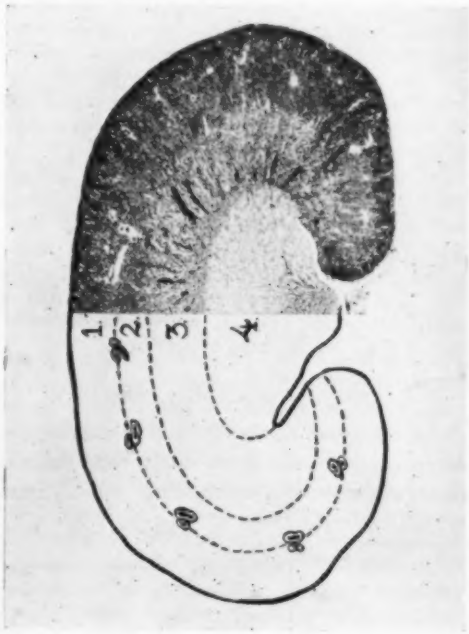
Na^{22} per day has become fairly constant at the end of between 48 and 72 hours.

Table 2 is a collection of data on 24-hour urinary excretions of sodium and potassium from typical control and sodium-deficient animals. Data are included to show results obtained during a preliminary period with the animal on the control diet as well as following the change to the sodium-deficient diet. The latter data are included to demonstrate the rapidity with which the rat kidney adapts to changes in intake of sodium.

In evaluating the exposed areas on the films, it is necessary to consider the usual architecture of the rat kidney. Fortunately, it can be divided into four specific zones, each of which contains a predominant tubular constituent.[9] Figure 3 is a hemisection of rat kidney showing these zones. Zone 1 extends from the level of the arcuate arteries to the periphery of the cortex and contains chiefly proximal convoluted tubules. Zone 2, predominantly straight portions of proximal convoluted tubules, extends from Zone 1 to the edge of the medulla. Zone 3 is the outer stripe of the medulla and contains chiefly the ascending limbs of Henle's loops and the vasa recta. Zone 4 is the papilla and consists predominantly of collecting ducts. Table 3 is adapted from McFarlane[9] and is a summary of the components of each zone. The predominant tubular constituents are indicated by asterisks.

The autoradiographic patterns pictured in the various figures have been interpreted on the basis of the presence or absence of exposure in the various zones and, where possible, on the relative intensity of this exposure. These findings were then correlated with several other factors: namely, plasma and urine levels of Na^{22}, variations in experimental procedure and known actions of sodium under the particular circumstances.

Fig. 3.—Horizontal hemisection of rat kidney. The zonal distribution of the tubular constituents is clearly discernible in the upper half of the figure. These zones, the contents of which are listed in Table 3, are shown diagrammatically in the lower part of the figure.



It must be emphasized that no attempt was made at precise quantitative evaluation of the amounts of sodium present in any portion of a section. It was found, however, that rough approximation of relative amounts of Na^{22} in the various zones could be made by means of serial exposures. The procedure consisted of exposing a series of sections from each preparation for varying lengths of time. By this means, the zone first showing the presence of radioactivity could be said to have the highest amount of Na^{22}, with succeeding appearance indicating less amounts.¶ An additional factor taken into account in all interpretations was the level of the section in relation to the whole organ. Comparison was made with the stained histological section to correlate the relative amounts of each of the zones present in the corresponding autograph. A slice of tissue taken from a more peripheral area of the kidney has a different proportion of the various components than one taken in the exact mid plane.

TABLE 3.—*Distribution of the Epithelial Components of the Rat Kidney*

Zone 1	
Proximal convoluted tubules *	
Distal convoluted tubules	Outer
Ascending limbs of Henle's loops	cortex
Collecting ducts	
Zone 2	
Straight portion of proximal tubules *	
Ascending limbs of Henle's loops	Inner
Collecting ducts	cortex
Zone 3	
Ascending limbs of Henle's loops *	
Narrow limbs of Henle's loops	Outer
Collecting tubules (vasa rectae are concentrated in this zone)	medulla
Zone 4	
Collecting ducts *	Inner
Narrow limbs of Henle's loops	medulla

* Indicates major constituent of each zone.

Vascular Pattern Experiments.—The two groups of experiments described under Methods were designed to provide an index of the normal vascular pattern of the rat kidney as seen by this method. On the fair assumption that only minute amounts of P^{32} were present outside the red blood cells, the typical autograph represented in Figure 4 shows that the major vascular components are confined to Zones 1 and 3. Zone 2 shows characteristically little or no radioactivity and Zone 4 is pale compared with Zones 1 and 3. The large amounts of P^{32} apparent in Zone 3 are indicative of the concentration of blood vessels (vasa recta) in this area. In Zone 1 the picture represents a combination of blood in the arcuate vessels, glomeruli, and capillaries surrounding the proximal and distal convoluted tubules. It is of interest that, according to these experiments, there is only a small amount of blood present in the medulla, exclusive of the outer stripe.

In the series using Na^{22}, the renal pedicles were clamped at intervals of from 15 seconds to 5 minutes after the start of the intravenous injection of 5μc of Na^{22}. By this means, a progression of events was observed. There was, at first, a build-up of concentration in Zone 1 (Figure 8), which was most pronounced in the sodium-deficient animals. Zone 2 showed no appreciable exposure above background. At between 15 and 30 seconds, Zone 3 became faintly visible and, by this time, Zone 4 was almost entirely black and of equal intensity with Zone 1 (Figure 9). These pictures are not parallel to those with P^{32}, in that Zone 4 is darker and undoubtedly represents an additional contribution from extravascular Na^{22}. This is also the case in Zone 1, where there is glomerular filtrate containing the radioactive material in addition to the extracellular fluid. These findings are believed to show that there is an equilibration between plasma and extracellular fluid in the kidney within a period of one minute. Although the plasma level of Na^{22} is falling exponentially during this series, the extracellular level is rising so that the radioautographic situation is rela-

¶ It should be noted that the blackening of the exposed film is an index of absolute amounts of radiosodium on one portion of the tissue and has no relation to concentration of sodium. Thus, for example, an area of 1 cm.^{2} containing 1 ml. of fluid with a concentration of 100 mEq Na^+/liter would give a greater exposure than 0.1 ml. of fluid in a similar area containing 500 mEq Na^+/liter.

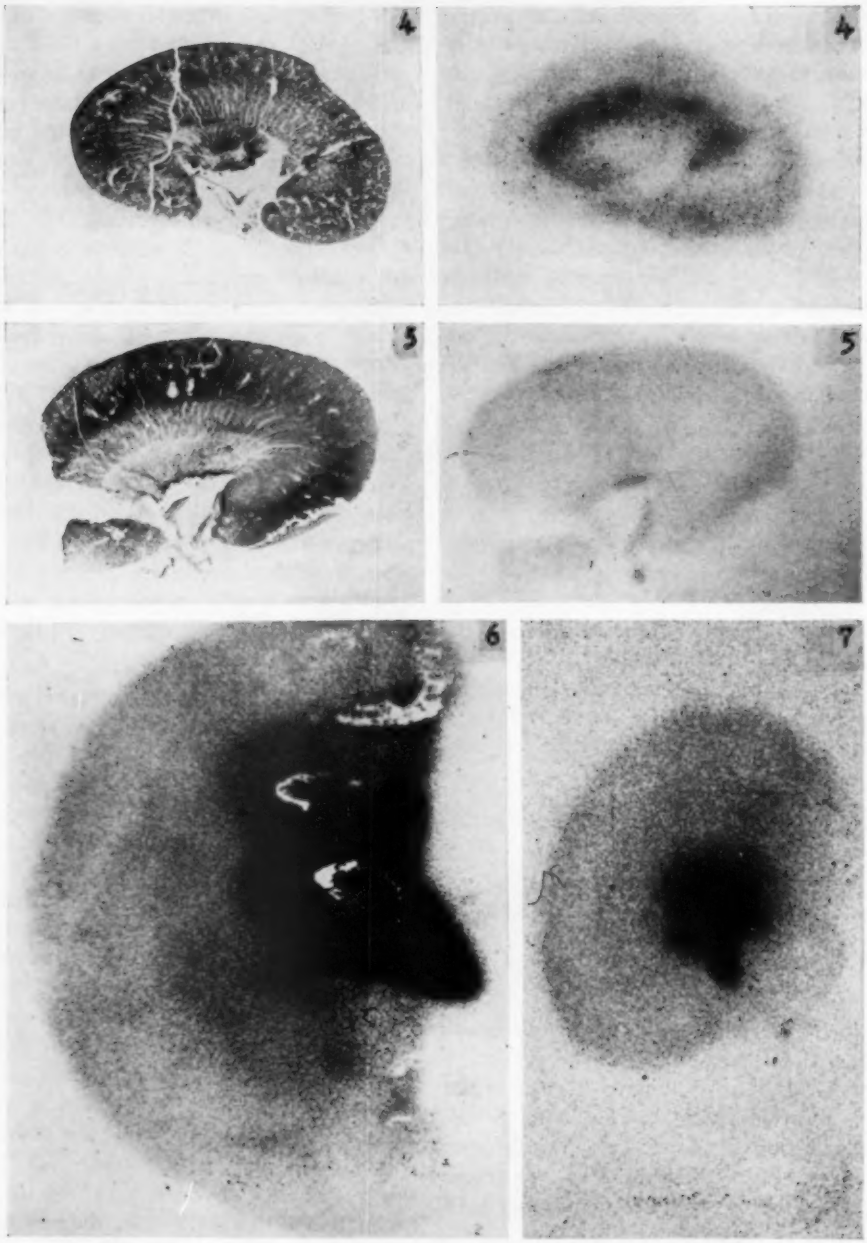


Fig. 4.—Radioautograph and stained section (toluidine blue, yellow filter; mag. × 4.5) of rat kidney following intravenous injection of P^{32} labeled red cells. Animal No. 66 L. B.

Fig. 5.—Radioautograph and stained section (toluidine blue, yellow filter; mag. × 4.5) of kidney from rat on control diet 30 seconds following intravenous injection of Na^{22}. Animal No. 54; 30 seconds, C.

Fig. 6.—Radioautograph of kidney from a rat on control diet five minutes after intravenous injection of Na^{22}. Note marked exposure in Zone 4. Animal No. 10; five minutes. Mag. × 7.

Fig. 7.—Typical radioautograph from a normal animal, regardless of previous diet, at any period five minutes to 24 hours from the time of injection of Na^{22}. Animal No. 44; 4 hours, No. 912. Mag. × 5.

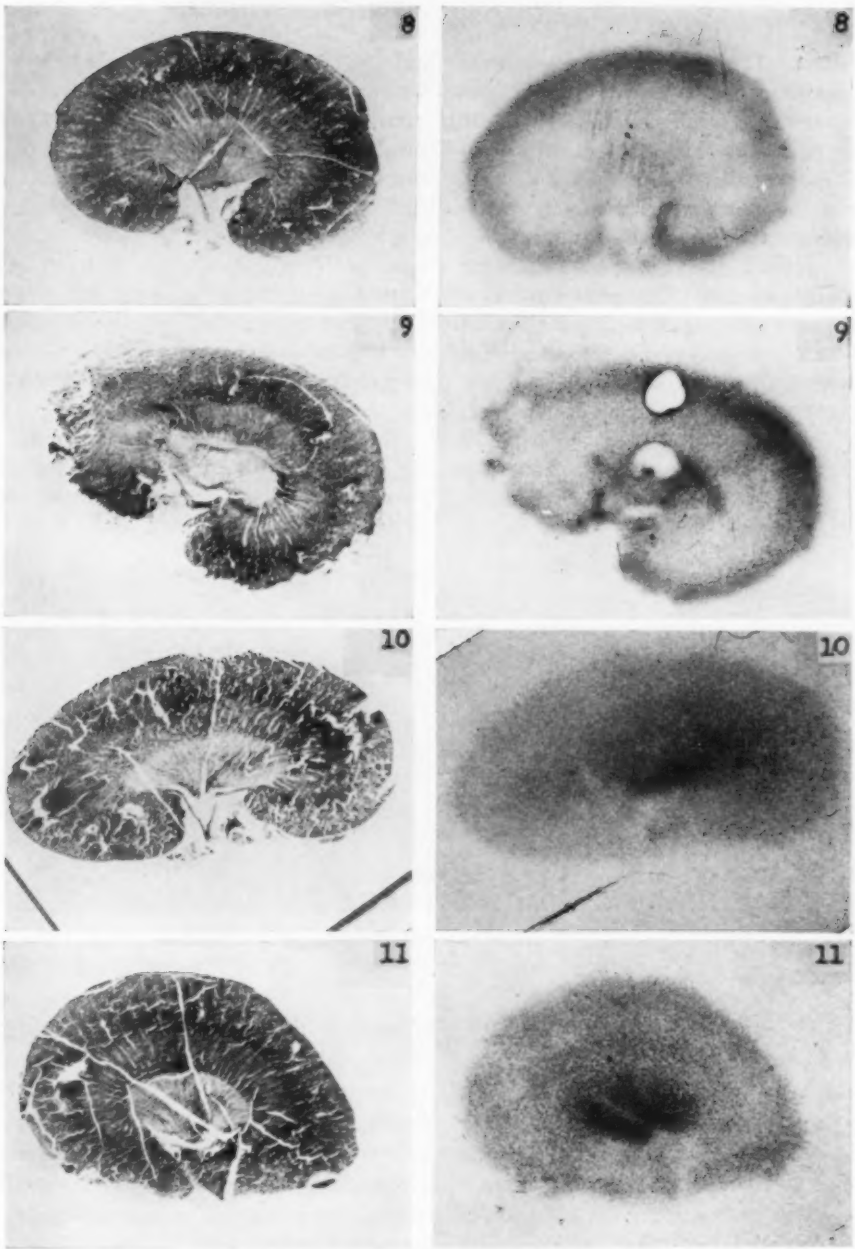


Fig. 8.—Radioautograph and stained section (toluidine blue, yellow filter; mag. × 4.5) of kidney from rat on the sodium-deficient diet removed 15 seconds following intravenous injection of Na^{22}. Animal No. 48; 15 seconds, E.

Fig. 9.—Radioautograph and stained section (toluidine blue, yellow filter; mag. × 4.5) of kidney from rat on the sodium-deficient diet removed 30 seconds following intravenous injection of Na^{22}. Animal No. 48; 30 seconds, E.

Fig. 10.—Radioautograph and stained section (toluidine blue, yellow filter; mag. × 4.5) of kidney from rat on the low-sodium diet 1.5 minutes following intravenous injection of Na^{22}. Animal No. 49; 1.5 minutes, A.

Fig. 11.—Radioautograph and stained section (toluidine blue, yellow filter; mag. × 4.5) of kidney from rat on the low-sodium diet two minutes following intravenous injection of Na^{22}. Animal No. 50; two minutes, C.

tively static. The contribution from urine or tubular fluid to the exposure in the medulla is certainly negligible in these early films owing to the small amount, if any, of filtrate reaching this region. An additional feature of this group may be seen when the autographs, representing the condition at between 30 seconds and one minute following injection into the two different preparations, are compared. Figure 9 is taken from an animal on a low-sodium diet and shows the characteristic pale Zone 2, in addition to the findings mentioned above. Figure 5, however, from a control animal at the same interval (30 seconds) shows Zone 2 to have definite exposure, which may be related to the fact that these animals were definitely

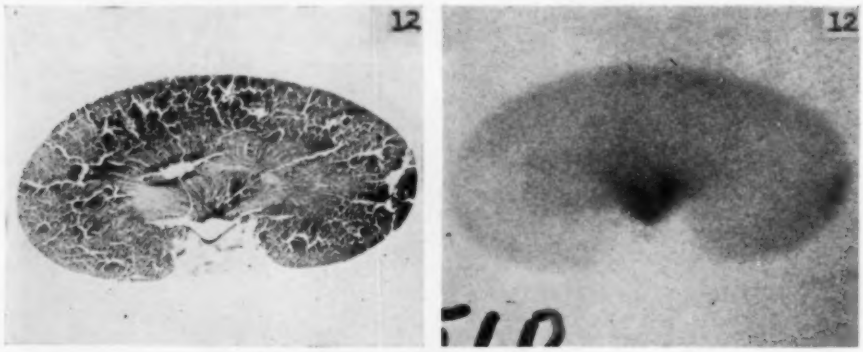


Fig. 12.—Radioautograph and stained section (toluidine blue, yellow filter; mag. × 4.5) from kidney of a rat on the sodium-deficient diet 72 hours after intraperitoneal injection of Na^{22}. Animal No. 51; 72 hours, D.

in saline diuresis inasmuch as 1 cc. of 0.9% NaCl had just been injected.

The characteristics of the medullary exposure change abruptly at an interval of between two and four minutes following the injection. At this time, Zone 4 suddenly becomes much blacker than Zone 1, especially in the sodium-deficient animal (Figs. 6, 10, and 11).

Normal Rat.—The autographs prepared in the study of vascular patterns served as the basic unit for experiments with both control and sodium-deficient animals. The next group to be studied was one in which animals were killed at periods of from 30 minutes to three days following intraperitoneal injection of Na^{22} in doses of 5μc and 10μc. As shown in Figure 1, the plasma level of injected material is in an equilibrium condition which it maintains for approximately four hours. Although both dosage levels had parallel curves, they differ in that radioautographs prepared from the animals which received 10μc of Na^{22} showed a prominent Zone 3 while those receiving 5μc did not. These data served, therefore, as an index of the amount of intravascular Na^{22} necessary to give appreciable exposure in that area of the section.

The essential findings in this group are the following: (*a*) Zone 1 is black, owing to the combination of exposure from the radioisotope in the plasma, extracellular fluid, and glomerular filtrate; (*b*) Zone 2 is pale, in sharp contrast with Zone 1; (*c*) Zone 3 is apparent in the higher dosage levels and faint in the lower, and (*d*) Zone 4 shows much greater exposure than any of the other areas, as determined by the method described above. An autograph representative of this series is identical with Figure 7. Since the plasma forms so small a constituent of the medullary fluids, as demonstrated with P^{32}, the source of the exposure in Zone 4 must be from extracellular fluid, tubular fluid, and urine, and possibly the cells of the collecting ducts. Even though there is possibly more plasma and extracellular fluid per unit area of the medulla, the absolute quantity present

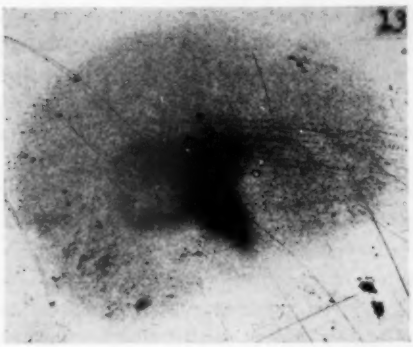


Fig. 13.—Radioautograph of a rat kidney following injection of a diuretic dose of organic mercurial. Animal No. 33 (4.8 mg. of Hg per kilogram of body weight as meralluride four hours before killing); 10 μc Na^{22} intraperitoneally 30 minutes before killing; slide No. 795. Mag. × 5.

in each section is very small. These autographs, therefore, are believed to indicate that there is a considerable amount of emission from tubular fluid and urine or collecting duct cells.

The long-term experiments show this even more strikingly. In these, the animals were killed three and four days after the intraperitoneal injection of 10μc of Na^{22}. Figure 1 shows that the plasma levels had fallen to a point at which even extracellular fluid concentrations could barely be detected in the autograph. There was, however, still considerable exposure in Zone 4.

Sodium-Deficient Rat.—The experiments using these animals were arranged to parallel the control series. On the whole they showed no great differences, discernible by this method, from the normals. Identical procedures showed variation only insofar as Zones 1 and 4 were always blacker than the normals in the same elapsed periods of time, even though the plasma levels were not appreciably higher in this group in the early stages of the experiments (Fig. 12).

Effects of Pharmacologic Agents.—Corticotropin, cortisone, desoxycorticosterone acetate, vasopressin injection, acetazoleamide.

The autographs obtained following the administration of the usually effective dosages # of these agents revealed no detectable difference from controls on similar dietary regimens. Animals were always killed at the time of expected maximum action of the drug. Plasma levels of Na^{22} obtained at the

Corticotropin (ACTH [Armour Laboratories]), two doses of 0.3 mg. per kilogram of body weight I. P. in a 24-hour period.

Cortisone acetate (Merck & Company, Inc.), two doses of 0.15 mg. per kilogram I. P. in a 24-hour period.

Desoxycorticosterone acetate (Doca; Percorten [Ciba]), 4 to 5 mg. subcutaneously per 200 gm. rat.

Vasopressin injection (Pitressin [Parke, Davis & Company]), 50 to 100 mU I. P. per 200 gm. rat.

Acetazoleamide (6063 [American Cyanamid Co.]), 200 mg. per kilogram of body weight I. P.

TABLE 4.—*Summary of Organic Mercurial Experiments*

Animal No.	Diet	Dosage and Type of Mercurial	Survival Time (in Hr.)	Na^{22} Dose	Time Pedicle Clamped (in Min.)	Killing	Exposure by Zones 1	2	3	4	Misc.
34	Control	2.4 mg. Hg/kg. (meralluride)	4.0	5μc I. P.	30	K	+	+	+	++	.....
33	Control	4.8 mg. Hg/kg. (meralluride)	4.0	10μc I. P.	30	K	+	+	+	++	See Fig. 13
57	Control	5 mg. Hg/kg. (meralluride)	0.5	5μc I. P.	30	K	+	+	+	++	.....
30	Control	5.0 mg. Hg/kg. 3 times in 24 hr. (meralluride)	27.3	5μc I. P.	45	K	+	++	+	+	Zone 2 necrotic See Figs. 14 and 15
17	Control	48 mg. Hg/kg. (mersalyl [Salyrgan] sodium and theophylline)	5	5μc I. V.	3 and 6	*	++	++	+	+	Zone 1 and 2 necrotic

I. V. indicates intravenous injection; I. P., intraperitoneal injection.

Radioautographic data are given in exposure by zones as: — indicates no exposure of film above background; ±, faint exposure above background; +, definite exposure of film; ++, region of greatest exposure (determined as described in text).

Time pedicle clamped is taken from the start of the Na^{22} injection. K indicates killed by blow to head; *, kidneys removed from living, anesthetized animal.

killing were almost identical with the controls. Since these were acute experiments, changes in urinary excretion could not be determined.

Organic Mercurial Compounds.—The dosage of meralluride (Mercuhydrin) used was based on the work of Brown and Englebach.[10] Calculations were made on the basis of milligrams of mercury in the compound rather than of the weight of the organic molecule. Two dosage levels were used: the first, known to give a diuresis in rats, and a second, which was twice the diuretic dose and was repeated at 8-hour intervals for 24 hours (Table 4). In each case, the autographs showed a distinct departure from the normal. All animals were killed 0.5 to 5 hours after the last injection of mercurial. The tracer material was given intraperitoneally 6 to 45 minutes before killing.

When the diuretic dose was used, the most pronounced change seen was a definite exposure in Zone 2, a phenomenon observed only in osmotic diuresis and in the early stages of saline diuresis. Zone 4 was much blacker than any of the other areas, as in the controls (Fig. 13). The stained sections from all of these kidneys were examined with special care to rule out the presence of any tubular lesion. None was found.

Kidney sections (Fig. 14) of the group which received repeated toxic dosages of organic mercurial showed marked necrosis of the straight portion of the proximal convoluted tubules (Zone 2). These findings are similar to the characteristic picture produced when minimal, but toxic, doses of mercuric bichloride are administered.[11] The autographs obtained from these kidneys showed an even diffuse exposure throughout all portions of the section (Figs. 14 and 15). Most remarkable, however, was the finding of a dense ring of exposure exactly coincident with the necrotic area in the parent section and the absence of the usual predominant exposure in Zone 4. The findings in Zone 2 coincide with the localization of Hg^{203} in the kidney observed by Lippman.[12]

Diuresis Experiments.—One additional group of experiments involving osmotic diuresis is described in the following section.

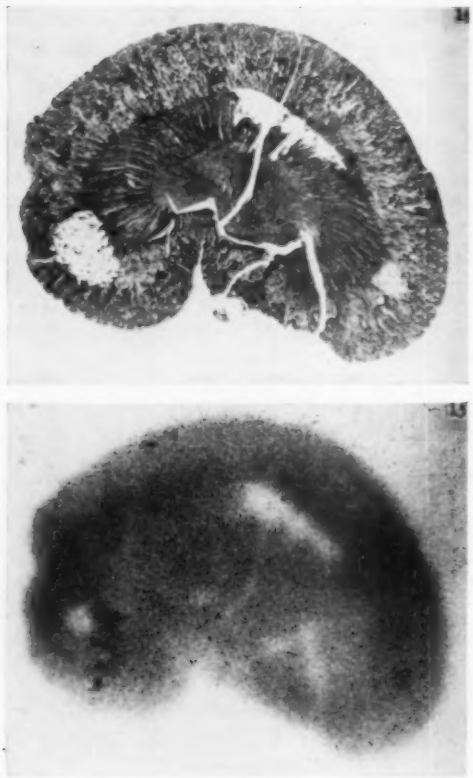


Fig. 14.—Section of rat kidney from animal No. 30 which received 5 mg. of Hg/kg. of body weight as meralluride twice in 24 hours. Note the necrosis of the proximal tubule in Zone 2. The irregular margin of the border between Zones 1 and 2 is clearly demarcated in this photograph, as the unstained necrotic tubules are the straight portions of the proximal tubules and belong in Zone 2. The tears in the section are artifacts which occurred during cutting. Toluidine blue stain, yellow filter; reduced 4/5 from mag. × 7.

Fig. 15.—Radioautograph corresponding to the stained section shown in Figure 14. Note the even exposure throughout the section except for the ring of increased exposure in Zone 2. Reduced 4/5 from mag. × 7.

COMMENT

The rat was chosen as the experimental animal in these procedures for several reasons. The size of the kidney makes it especially suitable for use in the preparation of frozen and dehydrated sections, in that the entire organ easily fits on the slide. In addition, the well-defined architectural pattern offers an advantage in interpretation. Meta-

bolically the rat responds very quickly to changes in dietary intake, and the urinary sodium levels drop almost immediately in response to the removal of sodium from the diet (Table 2). Among the disadvantages of using this animal in experiments such as these are that unusual care is necessary in obtaining urine samples and only small amounts of blood are available for analysis except in acute experiments. Also, coincident renal function testing, which would have been desirable, was not possible. However, it is relatively simple to draw enough blood for radioactivity determinations in plasma by collecting 0.2 to 0.3 ml. of blood, from the tail, into miniature hematocrit tubes.

The radioautographs shown in the various figures are discussed on the basis of the zones as previously outlined. They represent conditions as they existed at one instant in the life of the kidney of the particular prepaation under discussion. In common with other investigators, we have not found a suitable means for more exact localization in an organ such as the kidney. The geometry of the exposure is such that the source of beta rays causing it cannot be determined with accuracy. Our conclusions of whether the source is parenchymal cells, blood vessels, extravascular fluid, or tubular fluid are based on a predominance of a type of tissue, the known behavior of sodium and chloride as determined by micropuncture techniques,* and variations in zonal distribution under different experimental conditions. The sharp lines of demarcation among the various zones speak for the implied unity.

Definition of the zonal distribution of kidney components, as made by McFarlane [9] after Peter,[15] seems to be borne out by our radioautographs. In addition to the predominant epithelial components, we have considered also the vascular and interstitial elements, since it is in these that one would expect to find most of the sodium ions. Here, too, the sharp radioautographic patterns seem to be indicative of unique function in each zone. This is seen in other types of metabolic experiments as well.†

Vascular Pattern.—These experiments were designed to demonstrate the exposure due to Na^{22} in its usual compartment, the extracellular fluid. In order further to separate this into intravascular and extravascular fluids, the experiments described with P^{32} were undertaken. The latter demonstrate that, radioautographically, the cortex and outer stripe of the medulla contain the most blood and that a smaller amount is present in the inner medulla. Comparing these findings with results from similar experiments with Na^{22} where the medullary exposure is greater shows that the extravascular fluid volume in this part of the kidney is considerable. Ljungberg [17] has calculated that in the rabbit kidney (which is structurally similar to that of the rat) the distribution in the medulla is as follows: collecting ducts, 5.5%; tubular epithelial cells, 14%, and interstitial tissue, 55% of the total. The fact that the Na^{22} in the cortex is from several sources, including glomerular filtrate, rules out any statement of activity with regard to the proximal or distal convoluted tubules, as determined by changes within the outer cortex (Zone 1). Similarly, since the exposure in Zone 3 is a function of plasma level of isotope, this type of experiment allows no conclusion as to the activity of the ascending limbs of Henle's loops, which form the largest epithelial component of this region.

The absence of exposure in Zone 2 in all but a few of the experiments is correlated with the known fact that this region in rodent kidneys does not contain appreciable amounts of blood, as determined by various injection techniques.‡

The pattern in Zone 4 is such that in the first minute following injection it is identical in exposure characteristics with Zone 1. It is only following this that the pattern changes, and we think that the radioautographs of the first 1- to 1.5-minute period represent the characteristic picture for

* References 13 and 14.

† References 16, 26, and 27.

‡ References 18 through 20.

sodium in the blood and interstitial tissue in the medulla.

Normal Pattern.—Comparing the autographs from intravenous injection with those seen following intraperitoneal injection shows only the previously described differences in Zone 3. In these experiments, the equilibrium was maintained for approximately four hours (Fig. 1). Manery and Bale [21] have shown by direct analyses that the percentage of the injected dose in the plasma remains constant for this period of time, sodium being absorbed from the peritoneal cavity at about the same rate as it leaves the circulatory system. Assuming complete diffusion in 30 minutes, extracellular fluid volume, as calculated by sodium space, showed average values of 29% for the control and 36% for the sodium-deficient animal in our series. These data are in agreement with those of other workers [8] who have studied this aspect of the problem more thoroughly.

Extending the preceding observations further, these radioautographs show the following approximation of events: Sodium, on entering the kidney, is rapidly diffused into the interstitial tissue and reaches equilibrium with the plasma in all portions of the kidney within two minutes. There being so little blood in the region of the straight portions of the proximal tubules (Zone 2), this area shows only slight exposure. In addition, the removal of some 80% of the sodium from the glomerular filtrate in the proximal convoluted tubule leaves too little to be seen radioautographically as the fluid traverses the tubules in Zone 2. For the reasons given above, these data permit no further evaluation of events along the course of the nephron until the medullary portion is reached. At this level, there appears, approximately 1.5 minutes after the Na^{22} reaches the renal arteries, a gradual but noticeable increase in the exposure from the medulla. This could represent one of three factors: (*a*) The tubular fluid, now concentrated into only a few collecting ducts, has reached the medulla and contains sufficient Na^{22} to cause the increase in exposure; (*b*) a slower diffusion component, that of Na^{22} moving into the cells of the collecting ducts, is now apparent, or, (*c*) a combination of both of these factors.

Proceeding on the assumption that the augmentation in exposure is from tubular fluid, it must be supposed that very little of the sodium leaving the most proximal portions of the nephron has been reabsorbed at any site above the collecting ducts. If, as has been shown by Dicker,[22] less than 3% of the filtered sodium reaches the urine in the rat and if the 97% is reabsorbed prior to the entry of the filtrate into the collecting ducts, the absolute amount reaching the medulla could hardly account for the marked increase seen in the autographs. The alternative, therefore, is the assumption that a fraction larger than 3% remains in the tubules at the collecting duct level.

On the other hand, the second explanation, that we are seeing the entry of sodium into collecting duct cells, would fit the observed facts. This could be a corollary to the situation in the distal portion of the nephron of frogs.[23] In either case, the results seem to show that the collecting tubules are permeable to sodium and that they may be implied to have reabsorptive and, possibly, secretory powers. Additional evidence for this is seen in the experiments on sodium-deficient animals. This group showed an even more pronounced exposure in the medulla than did the control at the three-minute period following injection. On the basis of the foregoing, it may be that the collecting duct cells normally containing sodium have been relatively depleted. When sodium is administered, the absolute amount of isotope taken up by these cells will be greater, and the resulting picture should show the increased uptake observed. In support of this, Greenberg, Campbell, and Murayama [8] have found that, in the sodium-deficient animal, there is an increase in apparent extracellular space in the kidney, as determined with Na^{22}. This is greater than that seen in any other organ except stomach.

This finding does not mean that the collecting ducts are the only site of the increased reabsorption of sodium in the nephron of the depleted animal. Comparison of the radioautographs taken 15 and 30 seconds after intravenous injection in normal and sodium-depleted animals (Figs. 5, 8, and 9) indicates that the proximal convoluted tubules also play a major role. The normal animal receiving 1 ml. of 0.9% NaCl intravenously is in the midst of a saline diuresis, and larger amounts of Na^{22} are therefore present in the straight portions of the proximal tubule. This is seen in the radioautographs (Fig. 5) from this group, in which Zone 2 is exposed to almost the same degree as is Zone 1. In similar experiments on the sodium-deficient animal, the usual finding of a very pale Zone 2 is apparent (Figs. 8 to 11). We interpret these to indicate that, in the presence of a previously low plasma sodium content, there is a pronounced stimulus to reabsorption in the proximal convoluted tubule. This does not, however, preclude equal or greater activity in the other sites mentioned above.

Ljungberg [17] demonstrated high concentrations of chloride in the medulla of rabbits by means of histochemical techniques. He postulated that the collecting duct system is involved in active chloride reabsorption. Smith [1] takes exception to this on the grounds that the available evidence indicates that it is more likely that sodium is the ion predominantly involved and that chloride is an indifferent anion. He speculates that the high intracellular concentration of ions in the collecting duct cells may be involved in the distal reabsorption of water to form a hypertonic urine. The findings presented earlier seem to show that in the sodium-deficient animal there is an augmented uptake of sodium in the collecting duct cells. It, therefore, seems that these cells are involved in an active reabsorption of sodium and, possibly secondarily, water. These statements are obviously inferences, but the interpretations are consistent with both our radioautographs and Ljungberg's chemical determinations. Also, Glimstedt and coworkers § reach a similar conclusion in a series of papers on nuclear volume changes in collecting tubule cells under varying experimental conditions.

Effects of Other Agents.—Effects of corticotropin, cortisone, desoxycorticosterone, and vasopressin injections, as seen by this method, have not been demonstrable. We have been unable to see any changes following administration of any of these in effective doses, either by means of radioautography or change in plasma levels of Na^{22}. These results are in accord with the postulates that the actions of these agents are probably slight exaggerations of normal mechanisms. As such, they are quantitatively beyond the measurements possible with this method. It is possible that certain refinements of this technique might provide the improved definition necessary for interpretable autographs.

The experiments using therapeutic doses of organic mercurial showed a distinctive pattern, in that it was one of the instances in which we saw a definite exposure from Zone 2 in animals in whom the Na^{22} was injected intraperitoneally. Figure 13 is a typical radioautograph taken from one of this group of kidneys. It shows the usual pattern, Zone 4 blacker than Zone 1, and, in addition, a prominent Zone 2, which is equal in exposure to Zone 1. This compares in some respects with the autographs from the animals in osmotic and saline diuresis. We interpret these radioautographs to indicate that the action of mercurials in producing natriuresis is in one of two sites. There is either an inhibition of reabsorption of sodium in the proximal convoluted tubules, thus allowing some of the ion to be seen in their straight portions, or there is an interference with a reabsorption process in the straight portions, not normally seen by our method, which becomes apparent under the influence of mercury. There is little likelihood that the mercurials exert any effect on the collecting duct system in Zone 4. This observation is suggested by the lack

§ References 24 and 25.

of marked exposure in Zone 4 in the experiments in which nephrotoxic doses of mercurial were used. The radioautographic pictures of Zone 2 in the diuretic experiments show a qualitative, but not quantitative, similarity to those animals in which nephrotoxic doses of mercurial were used. This may be an indication that the actions of these agents are related in the same degree and involve a specific change in the straight portions of the proximal convoluted tubules. Recent histochemical studies by Wachstein and Meisel [26] and Rennels and Ruskin [27] on the inhibition of succinic dehydrogenase by mercurials tend to support this idea. Mustakallio and Telkkä's [28] conclusion that the ascending limb of Henle's loop is the site of action of mercurial diuretics appears to have been invalidated by the above investigators.

The observations made when toxic doses of mercurials are given are incomplete, in that fluid balance studies were not made on these animals. The increased exposure in Zone 2 may be due to extracellular sodium entering the injured proximal tubular cells or, perhaps, it is due to back diffusion of glomerular filtrate through the injured cells into the interstitial tissue. The latter possibility would result in oliguria or anuria.[18] Studies on this problem are under way.

In addition to the mercurial, two other manifestations of diuresis were seen during the course of these experiments. One of these has been discussed earlier and consists of the observation that Zone 2 is exposed at about 30 seconds to one minute after intravenous injection of saline in the normal animal. This increased exposure was not seen when intraperitoneal injections were used. The sodium-deficient animals did not show this phenomenon. The second type seen was in the animals which received 10 ml. of 5% dextrose in water in addition to Na^{22} one hour before killing. The radioautograph obtained in this case was one of diffuse black throughout the entire section without any specific indication of zonal demarcation. These autographs are not shown because they photograph as a black image of a kidney section, without definition of anything but the boundaries of the slice. The observations is a proportionally decreased reabsorption in in all of these experiments indicate that there the proximal convoluted tubules under the influence of saline, osmotic, and mercurial diuresis. No conclusions can be drawn as to the stimulus for this phenomenon, as it may be changes in glomerular filtration rate, renal plasma flow, filtration fraction,[29] or the influence of humoral factors. The autographs obtained in two acetazoleamide experiments were identical with those from normal animals under similar conditions, except that Zone 4 showed somewhat greater exposure. On this basis, it appears that the site of natriuretic action of this potent carbonic anhydrase inhibitor in the kidney is not the proximal tubule. To that extent, it supports previous conclusions placing its diuretic activity in a more distal portion of the nephron.‖

Certain incidental findings, not previously described, are presented here. These were not pursued beyond the initial observations but are mentioned in order to indicate further uses for the radioautographic method which might be productive in studies of function.

In the course of the many experiments performed, it was inevitable that some of the procedures would not go according to plan. On several occasions, accidental perforation of blood vessels resulted in the animals' going into profound shock during the course of the experiment. The usual procedure was followed, out of curiosity, and in none of these instances of grossly visible shock were we able to demonstrate any sort of increased blood flow through the juxtamedullary circulation, neither was there ever any evidence of cortical ischemia. Although Na^{22} is not an ideal material for this type of experiment, it speaks against the presence of such a shunt in the shock state. Under the same conditions, identical results were obtained with P^{32}-labeled cells.

‖ References 30 and 31.

SUMMARY AND CONCLUSIONS

The use of radioautography as it applies to the study of renal metabolism of sodium has been presented. The validity of the various techniques used is discussed.

Our experiments permit the following conclusions:

Within certain limits, this method is suitable as an adjunct to other precedures for the localization of sodium in certain areas of the mammalian kidney.

The major vascular components of the rat kidney are in the outer cortex and the outer stripe of the medulla (vasa rectae). The region occupied chiefly by the straight portions of the proximal convoluted tubules contains a relatively small amount of blood, and the inner zone of the medulla is only slightly more vascular.

Equilibrium between intravascular and extravascular extracellular fluid within the rat kidney is reached within approximately two minutes, as determined with Na^{22}.

The uptake of Na^{22} in the kidney of a rat on a sodium-deficient diet is more rapid and markedly greater than that of the normal.

The high percentage of reabsorption of sodium in the proximal convoluted tubules is clearly seen by means of radioautography.

There is evidence of participation of the parenchyma of the medulla of the rat kidney in the metabolism of sodium.

The proximal convoluted tubule is involved to some extent in osmotic and saline diuresis, in that appreciable amounts of sodium are present in the straight portions of these tubules under these conditions.

A portion, if not all, of the action of organic mercurial diuretics in therapeutic dosages is in the proximal tubule.

Necrosis of the straight portion of the proximal tubule follows the administration of toxic doses of organic mercurial in the rat. Increased amounts of radiosodium are present in this region under such conditions.

Observable effects were not produced following the use of usually effective dosages of corticotropin, cortisone, desoxycorticosterone acetate, or vasopressin injection.

Acetazoleamide produces no discernible changes in the proximal tubular pattern as seen by this method, thus indicating a more distal portion of the nephron as the site of its diuretic action.

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Studies on the Viscosity of the Cytoplasm of Mouse Ehrlich Ascites Tumor Cells

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Heilbrunn and his collaborators have demonstrated a layer of rigid peripheral cytoplasm in certain nonmammalian cells * and have evidence that it depends upon calcium for its maintenance. They believe that changes in the sol-gel state of the cytoplasm, brought about by shifting of the cortical calcium, determine the response of cells to stimulation.†

Though Wilson and Heilbrunn [7] have suggested that all cells are endowed with a stiff cortex, evidence that such a cytoplasmic layer may exist in cells of mammalian origin has not been reported heretofore.

Using techniques similar, in part, to those of Heilbrunn and Daugherty,‡ we have demonstrated rigidity of the peripheral cytoplasm of cells of the mouse Ehrlich ascites tumor and have found that this rigidity can be altered by changes in the calcium content of the cytoplasm.

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* References 1 through 6.

† References 1, 7, and 8.

‡ References 1 and 8.

MATERIALS AND METHODS

The Ehrlich ascites tumor § was suited to this investigation because the cells can be obtained in isolated form without injury. Love and co-workers [9] described refractile lipid granules in the periphery of the cytoplasm of the tumor cells. Under the dark contrast oil immersion lens of a phase contrast microscope, these lipid granules appear as immobile bright spheres against the dark cytoplasmic background. They are located principally between a peripheral homogeneous and an inner granular mass of protoplasm (Fig. 1).

In the experiments reported here, the shifting of the lipid bodies under centrifugation was used as a measure of the rigidity of the peripheral cytoplasm. Ready movement of granules indicated diminished viscosity, while failure of inclusions to move indicated a firmer protoplasmic state.

The tumor was maintained by weekly transfers in male Strong A mice,‖ 3 to 6 months of age. For transfers, 0.2 cc. of the neoplastic ascitic fluid was injected intraperitoneally into each mouse. The animals were given a standard commercial diet ¶ and water freely.

For the performance of tests, the hosts were killed by severing the cervical spine 10 days after inoculation and ascitic fluid was aspirated into clean, dry 10-cc. syringes. Samples of 1 cc. were transferred promptly into serological tubes and test reagents were added. The tubes were sealed with Parafilm and refrigerated at 12 to 15 C for three hours. This three-hour interval was found most satisfactory for easily measurable and reproducible effects.

All equipment was washed with a detergent # and thoroughly rinsed with glass-distilled water. Cleaning reagents containing heavy metals were not used.

§ Supplied by Dr. Robert Schrek, of Hines Veterans Administration Hospital, Hines, Ill.

‖ Supplied by Mrs. Eleanor B. Harding, Department of Bacteriology, Northwestern University Medical School.

¶ Rockland Mouse Diet, Arcady Farms Milling Company.

Alconox, Inc., Jersey City 4, N. J.

RESULTS

A. Standardization of Procedure

Since changes in the viscosity of the tumor cells were to be determined by observing the effects of centrifugation in causing a shift of cytoplasmic granules, preliminary studies were necessary to establish standards for quantitative estimation of changes in granule mobility. After examining a number of specimens, we decided that no shift in granules would be considered to have occurred in a cell unless all the granules in that cell were found to be located at one pole, in an area no greater than one-third the total area of the cell (Fig. 2). Such cells we called "polar cells." Even in untreated cell suspensions, however, the granules of some cells are located only at one pole. Determination of a shift in granules consisted, therefore, of comparing the percentage of polar cells in uncentrifuged suspensions with the percentage present after centrifugation.

Table 1.—*Effect of Physiological Saline Solution and Centrifugation on the Distribution of Cytoplasmic Lipid Granules in Ehrlich Ascites Tumor Cells*

	No. of Animals	Mean Per Cent Polar Cells *	Mean Viscosity Value *
In uncentrifuged ascitic fluid..	10	16 ± 1.0	....
In uncentrifuged saline-diluted ascitic fluid.....	8	16 ± 3.2	1.00
In centrifuged saline-diluted ascitic fluid.....	25	17 ± 3.0	0.94

* For definition see text.

The mean percentage of polar cells in untreated ascitic fluid was determined in 10 tumor-bearing mice. A drop of ascitic fluid from each mouse was placed on a microscopic slide, covered with a cover slip, and viewed by dark phase contrast microscopy under the oil immersion lens. Five hundred tumor cells in random fields were examined. Polar cells were readily identified. All cell counts in this report were made in this way.

Clotting of ascitic fluid, which interfered with cell counts early in the study, could usually be prevented by dilution with an equal amount of physiological saline solution. The percentage of polar cells in such diluted suspensions was not significantly different from that in undiluted ascitic fluid (Table 1). In the remainder of the experiments reported here, therefore, all samples of ascitic fluid were diluted 1 : 1 with saline solution, except in experiment described under Section B5, below.

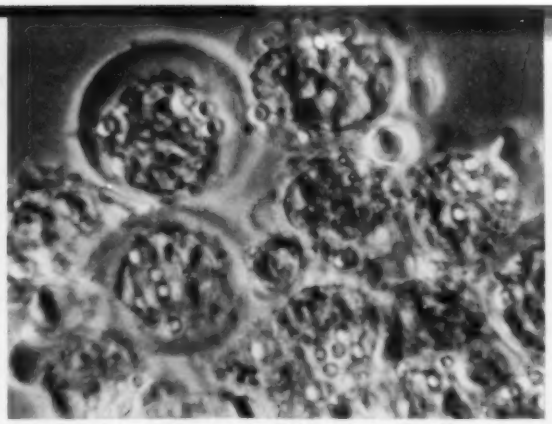

Fig. 1.—Untreated Ehrlich ascites tumor cells as viewed with the phase contrast microscope. Note the refractile lipid granules randomly distributed in the cytoplasm. Magnification × 1400.

The centrifugal force to be employed was determined by examining untreated cells after centrifugation at various speeds and for various periods of time. The tests were made with a Servall SS-1 centrifuge in a cold room at 0 to 3 C. Table 1 shows that the number of polar cells in saline-diluted samples from 25 mice was raised only 1% by centrifugation for 30 minutes at 9900×g (8800 rpm). This period and speed were chosen, therefore, for subsequent studies.

To simplify the expression of differences in viscosity before and after treatment, we have defined Mean Viscosity Value (MVV) as the ratio of mean per cent polar cells in unmodified ascitic fluid to mean per cent

Fig. 2.—"Polar cell." An Ehrlich ascites tumor cell after treatment with edathamil disodium and centrifugation. Note the localization of lipid granules at one pole of the cell. Magnification × 1400.

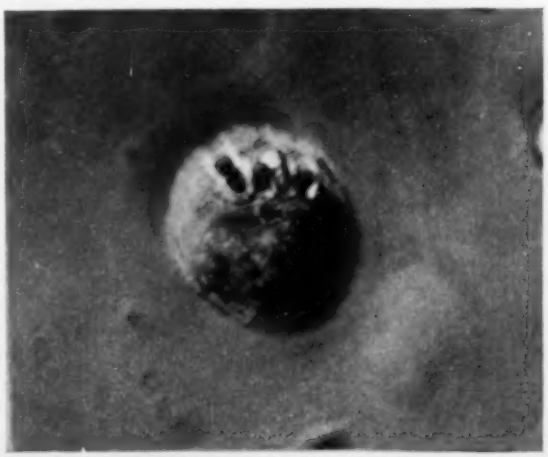

TABLE 2.—*Effect of Treatment of Ascites Tumor Cells with Calcium-Binding Agents*

No. of Mice	Agent	Concentration of Agent Added (mM/Cc.)	Mean Per Cent Polar Cells	Mean Viscosity Value
25	None *	.....	17 ± 3	0.94
7	Potassium oxalate	8.2×10^{-3}	30 ± 5	0.54
6	Sodium citrate	8.2×10^{-3}	26 ± 3	0.62
12	Edathamil disodium	3.0×10^{-4}	28 ± 4	0.57

* Saline-diluted control.

polar cells in centrifuged test suspensions. For example, the MVV of saline-diluted centrifuged cell suspensions is 16%/17%=0.94 (Table 1).

B. Experiments

1. *Effect of Calcium-Binding Agents.**—Aqueous solutions were prepared consisting of 0.27 M potassium oxalate, 0.27 M sodium citrate, and 0.024 M edathamil disodium (sodium ethylenediamine-tetraacetate; Na-EDTA).† These stock solutions were added to 2 cc. samples of saline-diluted ascitic fluid in amounts constituting 8.0×10^{-3} mM. of potassium oxalate or sodium citrate and 3.0×10^{-4} mM. of edathamil disodium for each cubic centimeter of diluted ascitic fluid sample. Untreated samples of the diluted ascitic fluid served as control. All samples were agitated briefly, sealed, and refrigerated for three hours. The suspensions were then poured into plastic centrifuge tubes (10×2.7 cm.) and centrifuged.

The marked reduction in protoplasmic viscosity of the tumor cells produced by the calcium-binding agents is shown in Table 2.

2. *Effect of Combined Treatment with Calcium and Calcium-Binding Agents.*—Two experiments yielded evidence that the reduction in cell viscosity by calcium-binding agents was in fact the result of withdrawal of calcium from the cells.

(*a*) In the first experiment, cell suspensions were prepared as in Section B1, but a solution of calcium chloride was added simultaneously with the three calcium-binding agents to see whether the presence of excess calcium would prevent reduction of viscosity. The quantity of calcium chloride was in excess of the theoretic amount that the agents would bind.

(*b*) In the second experiment, cell suspensions were treated with a calcium-binding agent before the addition of calcium chloride to see whether replacement of calcium theoretically withdrawn by the agent would restore the cytoplasm to normal viscosity. Cell suspensions containing edathamil disodium were prepared and refrigerated as in Section B1. The cells were then packed by slow centrifugation (1500 rpm) and the supernatant was discarded. This supernatant presumably contained bound calcium as well as excess binding agent. To rid the preparation further of bound calcium and binding agent, the packed cells were washed and centrifuged twice in 0.85% NaCl. The cells were resuspended in 1 cc. of saline solution and 1 cc. of M/2 calcium chloride was added. After 15 minutes at room temperature, the cells were again twice washed and centrifuged at low speed. Finally the cells were resuspended in saline solution and the number of polar cells was determined after the standard high-speed centrifugation.

The mean viscosity values obtained in the two tests were the same as those in the untreated controls (Table 3).

TABLE 3.—*Effect of Combined Treatment of Ascites Tumor Cells with Calcium-Binding Agents and Excess Calcium*

No. of Mice	Agent	Concentration of Agent Added (mM/Cc.)	Concentration of Calcium Chloride (mM/Cc.)	Mean Per Cent Polar Cells	Mean Viscosity Value
25	None *	.....	.....	17 ± 3	0.94
6	Potassium oxalate	8.2×10^{-3}	2.5×10^{-1}	17 ± 2	0.94
5	Sodium citrate	8.2×10^{-3}	2.5×10^{-1}	16 ± 3	1.00
7	Edathamil disodium	3.0×10^{-4}	6.3×10^{-3}	16 ± 3	1.00
5	Edathamil disodium	3.0×10^{-4}	6.3×10^{-3}†	16 ± 1	1.00

* Saline-diluted control.
† Calcium chloride added 3 hr. after edathamil disodium.

* The term "calcium-binding" is used here to cover both chemical combinations of calcium with an anion and formation of a complex.

† Obtained from Eastman Kodak Company, Rochester, N. Y.

3. *Toxicity of Edathamil Disodium.*—Since the three calcium-binding agents behaved alike in reducing cytoplasmic viscosity, subsequent experiments were simplified by using only one of the agents. Edathamil disodium was chosen for further investigation. Although edathamil is known to have low toxicity for sea urchin eggs,[10] it was necessary, first, to determine its toxicity for the ascites tumor cells because it was possible that the observed viscosity changes were occurring in cells killed or damaged by the calcium-binding agents.

In one test, Schrek's technique [11] was employed to determine the effects of edathamil disodium on cell viability. Ascitic fluid was diluted with saline solution, edathamil disodium was added, and the preparation was refrigerated as in Section B1. The fluid was then gently shaken and diluted 1:10 with 0.05% eosin in Tyrode's solution. Two minutes later a sample was placed in a hemacytometer and the per cent of viable (unstained) cells was determined. Another sample of the same fluid was prepared in the same way except for the omission of the binding agent. Edathamil-treated samples from each of seven mice contained 94.9% ±3.8% viable cells and the corresponding untreated samples contained 94.5%±2.2% viable cells. As tested by this method, therefore, edathamil disodium was nontoxic for the tumor cells.

In a further test, edathamil-treated tumor cells were compared with untreated cells in respect to their ability to grow intraperitoneally in mice. The per cent of viable cells in an aliquot of ascitic fluid was determined as before. Additional samples of the same fluid were then prepared, with and without added edathamil disodium, as in Section B1. After the period of refrigeration, the fluid was diluted with sufficient saline solution to yield a preparation containing 1,000,000 viable cells in 0.2 cc. Sugiura[12] found that this number of cells is the minimum necessary to give growth in 95% to 100% of hosts. A group of 25 Strong A mice was injected intraperitoneally with this dose of untreated cells, therefore, and a group of 27 received the dose of edathamil-treated cells. The mice were weighed regularly after injection as a measure of the rate of development of ascites and the time of death was noted. The results of this rather rough test showed no significant differences between the two groups of mice. The mice injected with treated tumor cells gained weight slightly more rapidly than those injected with untreated cells, but, on the other hand, the time of death in the untreated-cell group was slightly earlier than that in the group injected with treated cells.

4. *Effect of Edathamil Disodium on Killed Cells.*—Although edathamil disodium was found to be nontoxic, it was possible, nevertheless, that its effect on viscosity was exerted chiefly or wholly on cells already dead or severely injured. This possibility was examined in tests of the effect of edathamil disodium on cells killed by heat and cold.

(*a*) Exposure of fresh tumor cells in a test tube to 100 C (three minutes in boiling water) did not change the appearance of individual cells under the phase contrast microscope, except for an increased granularity of the cytoplasm. When these cells were stained with eosin by the method of Schrek, all stained immediately, indicating that they were dead. The heat-killed cells showed no change in granule mobility when treated with edathamil disodium and tested by the standard centrifugation method.

(*b*) In another test, a tumor cell suspension was frozen at a relatively slow rate by packing in carbon dioxide snow. After storage in a freezer (—21.0 C) for three months, none of the cells was viable when thawed and tested by Schrek's technique and no growth occurred when they were injected intraperitoneally into mice. The viscosity of the cold-killed cells was not altered by edathamil.

It appears probable, therefore, that edathamil disodium acts principally on living cells.

5. *Effect of Other Cations on Edathamil-Treated Tumor Cells.*—It is conceivable that ions other than calcium, which may be present in the cell, are also important in

maintaining cell viscosity and that the effect of edathamil disodium is in part, at least, to chelate these ions as well as calcium ion. The activity of seven ions in relation to that of calcium in the pH range of tumor ascitic fluid was tested, therefore, in experiments like that described in Section B2a, except that saline dilution was made only in testing the effects of sodium ion. Table 4 shows the results obtained when chloride salt of Na^{+}, K^{+}, Mg^{++}, Fe^{++}, Fe^{+++}, Cu^{++}, and Mn^{++} were employed in an amount equal to that used in testing calcium. These ions failed to prevent edathamil from reducing cell viscosity.

It will be noted that under the conditions of the tests just described magnesium ion was ineffective. In a supplementary test, however, magnesium ion in a concentration of 0.25 mM. per cubic centimeter (40 times that used for calcium) showed moderate activity in preserving cell viscosity in the presence of edathamil disodium. The mean viscosity

TABLE 4.—*Effect of Combined Treatment of Ascites Tumor Cells with Edathamil Disodium* and Excess of Various Cations*

No. of Mice	Cation	Concentration Added (mM/Cc.)†	Mean Per Cent Polar Cell	Mean Viscosity Value
12	Sodium ‡	7.3×10^{-2}	28	0.57
4	Potassium	6.3×10^{-3}	28	0.57
5	Magnesium	6.3×10^{-3}	28	0.57
4	Manganese	6.3×10^{-3}	27	0.59
4	Ferric	6.3×10^{-3}	26	0.62
4	Ferrous	6.3×10^{-3}	24	0.67
3	Cupric	6.3×10^{-3}	25	0.64

* 3.0×10^{-4} mM/cc.
† Concentration of chloride salts of the cations.
‡ Physiological saline.

value in this test was 0.84, as compared with the value of 1.00 obtained with calcium ion in a concentration of only 0.0063 mM. per cubic centimeter.

6. *Demonstration of Calcium Distribution by Microincineration.*—The presence and location of calcium within the tumor cells were demonstrated morphologically by the combined use of microincineration and treatment with edathamil disodium. A white ash resulting from high-temperature microincineration indicates the presence of either calcium or magnesium but does not distinguish between the two.[13] To identify calcium as the principal source of white ash in the tumor cells, advantage was taken of the fact that at the pH of the tumor ascitic fluid (7.2 to 7.3, as measured with a potentiometer), edathamil disodium binds calcium but not magnesium.‡ Saline-diluted ascitic fluid was treated with edathamil disodium and refrigerated as in the experiment of Section B1. An untreated sample served as a control. The cells then were packed by centrifugation at low speed and the supernatant was discarded. After twice washing and centrifuging, the cells were resuspended in saline solution. A drop of the final suspension was placed on a clean slide and spread with

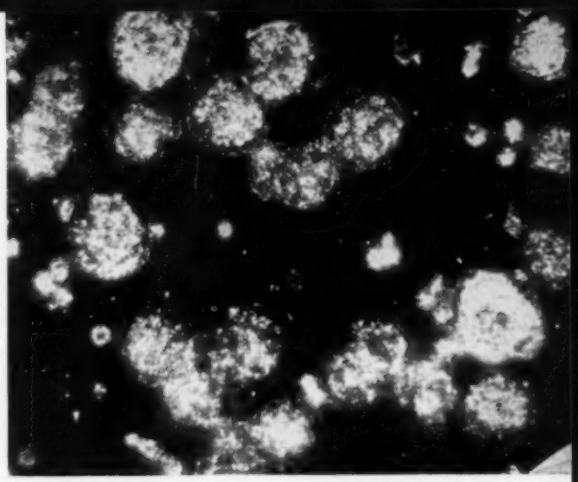

Fig. 3.—Untreated ascites tumor cells after microincineration, photographed by dark-field illumination. The white ash consists of oxides of calcium and magnesium. Magnification × 600.

‡ References 14 and 15.

Fig. 4.—Edathamil-treated tumor cells after microincineration. As compared with the untreated cells in Figure 3, the quantity of ash is greatly reduced. To obtain this print, the required exposure time was more than 5 times that employed in Figure 3. Magnification × 600.

the edge of another, as in the preparation of a blood smear. While the cells were still moist, the slide was immersed in isopentane chilled with liquid nitrogen and then dried under vacuum by the technique of Altmann-Gersh. The dried smear was ashed in a microincinerator at 600 C, as described by Scott.[16] The slide was then washed for 10 to 15 seconds in glass-distilled water at 80 C to remove soluble material. The insoluble material remaining on the slide included the oxides of calcium and magnesium. The preparation was then examined microscopically under dark-field illumination obtained with a cardioid condenser.

The untreated tumor cells showed a white, granular, halo-like outer cytoplasmic layer that varied somewhat in width. Just inside this rim was a clear area containing circular, empty spaces similar in position to that occupied by lipid granules in the living cell. The center of the cells was occupied by granular white ash, within which the indented and lobulated outline of the nucleus could occasionally be seen. In sharp contrast to the control cells, the outline of the edathamil-treated cells appeared as a thin, white veil, containing scattered bright dots, and the remainder of the cell structure was barely visible (Figs. 3 and 4).

COMMENT

In the studies upon which our experiments are based, Heilbrunn and his collaborators § used the movement of peripheral granules in centrifuged specimens of Amoeba proteus and the egg of the sea urchin, Chaetopterus pergamentaceus, as a measure of the viscosity of the cortical cytoplasm. They found that cortical viscosity was reduced by citrate and oxalate salts, presumably by withdrawal of calcium. Borei and Bjorklund,[17] using Versene as the binding agent, have confirmed this observation in the sea urchin egg. Heilbrunn found a similar reduction in cortical viscosity when cells were stimulated by ultraviolet light, x-rays, heat, cold, electrical impulses, or mechanical pressure.‖ These observations led him to propose that primary events in the response of a cell to stimulation are migration of calcium from the cortex to the center of the cell and concomitant liquefaction of the cortex and gelation of the inner cell mass.[18] This suggested migration of calcium has not been fully demonstrated, but the hypothesis is partly supported by reports that, in the aquatic plant Elodea, physical stimulation results in the appearance of crystals of calcium oxalate in central cytoplasmic vacuoles.¶

Of particular interest is the recent observation that a change in the sol-gel state of the cytoplasm as a result of stimulation (fertilization) precedes cell division in the Chaetopterus egg [4] and, further, that this change is prevented by Shear's polysaccharide and certain other polysaccharides, including heparin.#

In the experiments we report here, a number of the results obtained in nonmammalian cells have been duplicated in a mammalian tumor. Peripheral lipid granules in untreated Ehrlich ascites tumor cells failed to move except on prolonged centrifugation at forces above 10,000×g. Following Heilbrunn, we have assumed that this apparent fixation of granules is the result of high viscosity of the peripheral portion of the cell and we have described mobility of the granules in terms of relative viscosity. It should be recognized, however, that other factors may be important in determining the mobility of cellular inclusions in a centrifugal field. These include the density of cytoplasm relative to that of its inclusions, and the elasticity of cytoplasm. As Wilson has pointed out,[5] what is actually observed in experiments of this type is the dislodgment of cytoplasmic granules by shearing force. Hence, changes in the cytoplasm perhaps should be referred to only as changes in rigidity rather than in the specific quality viscosity.

As in the nonmammalian cells, treatment of tumor cells with cation-binding agents led to increased mobility of cytoplasmic granules.

§ References 1 and 7.

‖ References 1, 7, and 18.

¶ References 19 and 20.

References 21 through 23.

This mobilization occurred only in living cells and was not attended by diminished viability of the cells.

Subsequent experiments showed that the cation principally withdrawn by the binding agents was calcium. Thus, addition of an excess of calcium prevented the action of the binding agents in mobilizing the granules, whereas other cations, including magnesium, had no such effect when added in similar excess. Only in much higher concentration did magnesium show preventive action, and even then it was less effective than small amounts of calcium. Furthermore, high-temperature microincineration of untreated and edathamil-treated tumor cells demonstrated calcium in the outer cytoplasm.

We conclude that calcium, normally present in the periphery of the tumor cells, is responsible for the maintenance of the rigidity of this part of the cell and that a reduction in calcium content is followed by a change of the cortical cytoplasm to a more fluid state. It should be noted, however, that microincineration indicates the presence of calcium in the inner cell mass as well as at the periphery. The experiments reported here do not permit any conclusion either as to the viscosity of the inner mass relative to that of the periphery or as to the effect of withdrawal of calcium upon it.

It might be expected that in cells of mammalian origin, as in nonmammalian cells, an important relationship exists between sol-gel state and function. In preliminary observations, stimulation of ascitic tumor cells by exposure to ultraviolet light has reduced the rigidity of the peripheral cytoplasm—a result comparable to that described in amoebae. We have found, too, that in ascitic cells, as in the sea urchin egg, exposure to heparin and to Shear's polysaccharide diminishes cytoplasmic viscosity. Perhaps the proposal that polysaccharides prevent cell division in the sea urchin by their effect on sol-gel transformation can be extended also to the inhibitory action of Shear's polysaccharide on the growth of the Ehrlich ascites tumor and of certain other neoplasms.

SUMMARY

Tests of the mobility of cytoplasmic granules in a centrifugal field show that the peripheral cytoplasm of the mouse Ehrlich ascites tumor cell is rigid. In this respect, the periphery of the tumor cell may be compared with the cytoplasmic cortex described in certain nonmammalian cells.

The rigidity of the peripheral cytoplasm seems to depend principally upon the presence of calcium, which is shown by microincineration to be abundant in this zone. Withdrawal of calcium by binding agents (edathamil disodium [sodium ethylenediamine tetraacetate], sodium citrate, and potassium oxalate) results in diminished viscosity of the peripheral zone and, conversely, addition of calcium to tumor cells previously treated with a binding agent restores the zone to its normal state. Furthermore, of eight cations added in low concentration to tumor cell suspensions simultaneously with a binding agent, only calcium prevents a reduction in cytoplasmic rigidity. In much higher concentration, magnesium ion is moderately effective in countering the action of the binding agent.

These results indicate that the sol-gel state of the cytoplasm of a mammalian tumor cell is controlled by factors similar to those described in nonmammalian cells and suggest that a theoretically important relationship between sol-gel state and function in normal nonmammalian cells may exist also in neoplastic cells of animal origin.

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Pathologic Effects of Intravenously Administered Pyrogens on Large Animals

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As part of a study of the metabolism of tracer amounts of radioyttrium (Y^{91}) in laboratory and farm animals, histological changes in the lymph nodes, spleen, small intestines, liver, lungs, and, occasionally, the kidneys and brain were observed in cattle and sheep. Rhoades,* Heller,† Pierce,‡ De Bruyn,§ Margaret Bloom,‖ William Bloom,¶ and Murray # described the pathologic changes occurring in small laboratory animals administered Y^{91}, but none of the changes they saw resembled the lesions seen in the larger animals at this laboratory. Also, the small doses of Y^{91} used and the rapidity of the appearance of symptoms argued against a radiation effect. Therefore, further studies were made to identify the causative agent. The tissue distribution, placental transfer, excretion, and blood disappearance of the radioyttrium are being reported in other communications.*

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¶ Bloom,[1] Chap. 14, The Kidney.

Murray, R. G., in Bloom,[1] Chap. 7, The Spleen.

A. INITIAL OBSERVATIONS

Methods

In the first study, in which the prime interest was the distribution of radioyttrium, 9 cattle and 5 sheep were given 1.5 mc. per 100 lb. (45 kg.) of body weight † (Table 1). Two cows were pregnant at the time of injection and the resulting fetus and newborn calf were included in this study. The Y^{91} was radiochemically pure and carrier-free. It was administered by intravenous injection via jugular catheter [4] as a chloride in a solution of isotonic saline adjusted to 0.1 N HCl by the addition of NaOH. In some cases the dose was added to 40 ml. of homologous plasma before injection. Varying quantities of the physiological saline solution were used to wash the radioyttrium-containing solution from the administration apparatus. All distilled water used in this study came from a glass still which utilized tap water passed through an ion-exchange column.

After injection the animals were maintained in metabolism stalls for two weeks and then turned out to pasture, unless they were killed earlier. At selected intervals the animals were killed by stunning and exsanguination. Histologic samples were taken immediately and placed in a 10% formalin fixative. They were stained with a conventional hematoxylin and eosin method and examined microscopically.

Symptoms

In almost all animals there was apathy and anorexia lasting for a few days to several weeks following the injection unless they

* References 2 and 3.

† References 2 and 3.

TABLE 1.—*Animals from the First Study, with Their Age, Breed, Sex, Weight, and Time of Killing*

Animal No.	Age	Breed	Sex	Wt., at Injection, (Lb.)	Wt., at Killing, (Lb.)	Days After Treatment
Y-24	12 mo.	Hereford	Steer	838	838	1
Y- 4	13 mo.	Hereford	Steer	634	496	42
Y-31	20 mo.	Hereford	Steer	961	842	14
Y- 3	14 mo.	Hereford	Steer	696	730	60
Y-35	7 mo.	Guernsey	Bull	364	354	7
Y-36	6 yr.	Guernsey	Cow	811	710	7
Y-37	Fetus	Guernsey	Cow	...	40	7
Y- 1	23 mo.	Hereford	Steer	788	798	150
Y-48	7 days	Guernsey	Bull	...	68	97
Y-34	6 yr.	Guernsey	Cow	842	830	113
Y- 2	2½ yr.	Hereford	Steer	728	920	325
Y-38	98 days	Southdown	Wether	34	38	4
Y-39	90 days	Southdown	Wether	54	48	4
Y-60	82 days	Southdown	Ewe	54	52	24
Y-61	5 yr.	Southdown	Ewe	121	118	9
Y-56	5 yr.	Southdown	Ewe	204	207	111

were killed earlier. Weight loss, sometimes of considerable magnitude, was a frequent observation in the cattle. Even those in which there was a gain at the end of the period of observation had had a period during which their weight fell.

One steer (Y-4) had numerous enlarged external lymph nodes. This was so marked that a tentative diagnosis of lymphosarcoma was made and the animal was killed earlier than had been planned. Subsequent histo-

Fig. 1.—Small sinusoidal cluster of lymphocytes in the liver of a cow 24 hr. after receiving radioyttrium in distilled water intravenously (Y-24). It was initially thought that the lesion might have been associated with the radioactivity.

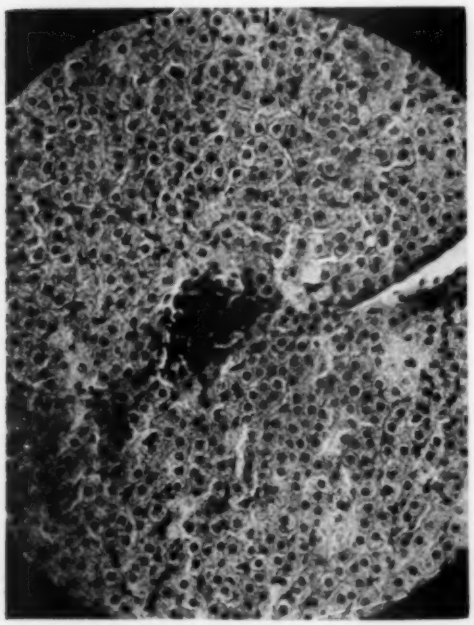
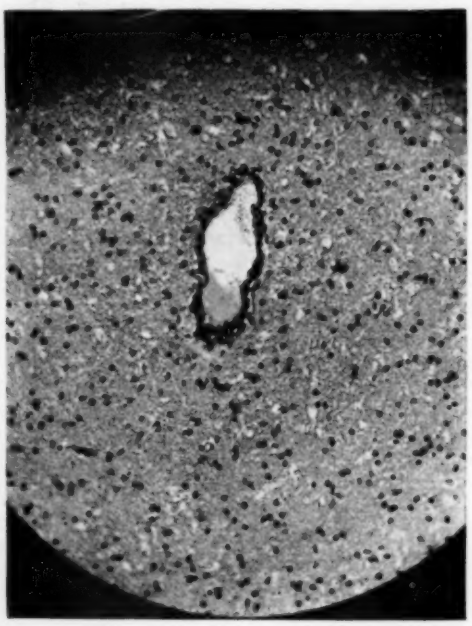


Fig. 2.—Perivascular cuffing in the cerebrum of a steer 42 days after having received radioyttrium in distilled water intravenously (Y-4). This animal had a severe lymphadenopathy at the time of killing. In this case, also, it was initially thought that there might have been a relationship between the lesion and the radioactivity.



logical examination did not support the diagnosis.

Gross Pathology

At autopsy no striking changes were observed, except in the single steer mentioned above. There was often mild to moderate lymph node enlargement, and such nodes had a pale cortex and a darkened medulla. Spleens were also larger than is usually anticipated in exsanguinated animals. The steer which was killed after the tentative diagnosis of lymphosarcoma had widespread lymph node enlargement. The spleen of this animal weighed 1250 gm., which contrasted to spleen weights of 300 to 500 gm. usually found in the normal animal of comparable size. There were many splenic abscesses, with great range in size. Subcutaneous and peritoneal fat had many petechial hemorrhages which frequently became confluent, forming large areas of hemorrhage. The myocardium was deeply hemorrhagic and the endocardial lining was lifted and distorted. The gall bladder was greatly enlarged, and the wall was thickened and intensely hemorrhagic on the inner surface. The liver was light tan in color, with several small, deep parenchymal abscesses and small petechial hemorrhages. The urinary bladder had one large subserosal hemorrhage. The lungs also had many small, deep and superficial nodules which when cut were caseous and resembled those seen in the liver and spleen. A few discrete areas of hemorrhage were noticed below the visceral pleura. The adrenal showed several small hemorrhages and there was a large marrow hemorrhage in a femur. The right eye had an extensive anterior chamber hemorrhage, plus some large petechiae in the periorbital fat.

Fig. 3.—A lymphocytic exudate about a small bronchial apparatus of a wether 4 days after injection of radioyttrium in distilled water intravenously. As in the other cases, no association between the lesion and the distilled water was made at this time.

Histopathology

All cattle and sheep given radioyttrium were unique in that they showed either a lymphocytic or, occasionally, a mixed lymphocytic-eosinophilic exudate of a granulomatous type in various tissues.

The intensity of the changes were related to some extent to the time after dosing at which the animals were killed, though even at 24 hours small lymphocytic foci would be found in the liver (Fig. 1) or heart. The exudate involved the lungs, liver, small intestines, myocardium, and lymphocytes most frequently. Occasionally the kidneys and brain were sites of pathologic changes. The severe leukemoid case mentioned above showed an exudate in the cerebellum and cerebrum (Fig. 2). This particular case was also unique in that there was a large diffuse subendocardial hemorrhage involving both the myocardium and the conduction fibers.

In all cases the germinal centers of the lymph nodes and spleen were reactive. The organ most usually affected was the lungs, where the animals always showed some lymphocytic exudate about small bronchial structures (Fig. 3) and alveolar septa. Lymphoid centers adjacent to small bronchioles were often prominent and reactive. In the leukemoid case the lesion was manifested as a microabscess (Fig. 4).

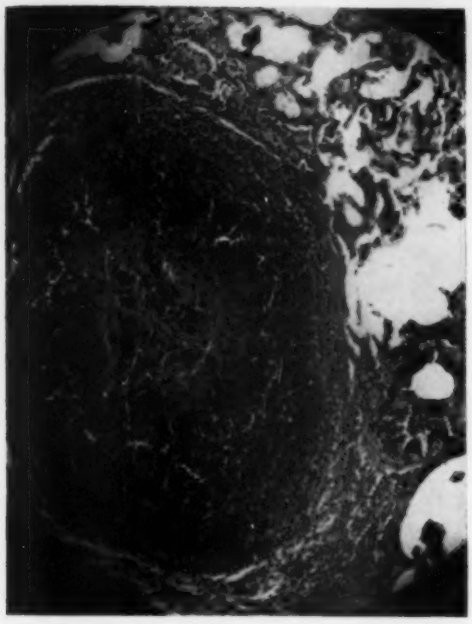


Fig. 4.—A microabscess in the peribronchial lymph node of a steer 42 days after having received radioyttrium in distilled water intravenously (Y-4). Figure 2 was also from this animal.

The liver, while it showed lesions most frequently, often was not so extensively involved as the lungs, except in the leukemoid case where many caseous microabscesses were seen. Granulomata in this organ were usually noted as small clusters of lymphocytes in the sinusoids or involving the portal triad. No relationship with the Kupffer cells could be established.

The exudate in the walls of the small intestines was different from that of the other tissues in that the exudate was of a mixed lymphocytic-esosinophilic type. The cores of the villi and valves of Kerkring were the principal centers of the lesion. The lamina propria, and sometimes the muscularis mucosae, were obscured by the large numbers of cells in the inflammatory process. Lymphoid nodules were often seen and were extremely reactive. In several cases there was a lymphocytic exudate of the myocardium. These were always around small venules or between the cardiac muscle fibers. The hemorrhage about the myocardium and conduction fibers in one case was mentioned above. One bovine fetus (Y-37) had more extensive heart (Fig. 5) and liver lesions than did the dam but did not have pulmonary or intestinal involvement. The newborn calf (Y-48) had no microscopic lesions in any tissue examined.

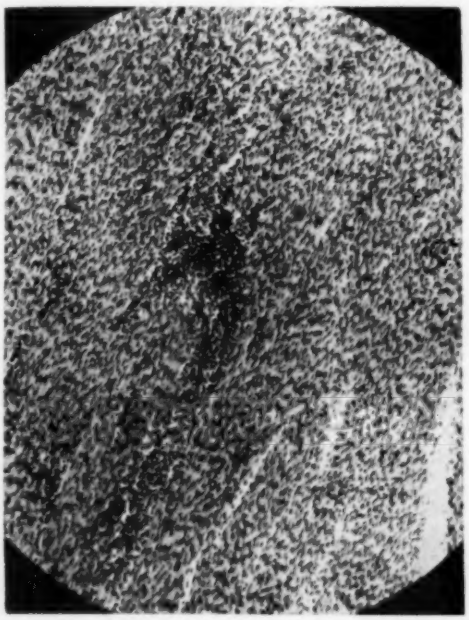
In all cases the granulomatous lesion noted was closely associated with vascular pathways or beds. It was so constant that it was worthy of special emphasis.

B. CONTROL STUDIES WITH SHEEP

Method

The observations reported above were unexpected and did not conform to any lesions customarily associated with internally deposited radioactive substances. A study was therefore designed, utilizing sheep, to determine if the changes seen were related to radioyttrium, stable yttrium, or possibly the vehicle. Five yearling ewes, two of which were pregnant, weighing from 90 to 112 lb. (41 to 51 kg.)

Fig. 5.—A lymphocytic exudate between the myocardial fibers of a bovine fetus which had radioyttrium, by way of its dam, 7 days prior to its killing while still in utero (Y-37). The dam had received the radioyttrium in distilled water intravenously. As in the other cases, it was initially assumed that there might have been a relationship between the radioactivity and the lesion. The uniqueness of this finding is that a pyrogenic substance can pass the placental barrier.



were selected and intravenously injected in accordance with the following dosage schedule:

(*a*) One animal: 5 ml. of 0.1 N HCl solution was taken, to which was added NaCl in quantity sufficient to make a 0.9% solution. This was then autoclaved at 17-lb. pressure for 60 minutes.

(*b*) One animal: NaOH added to 2.124 mc. of Y^{91} chloride in a quantity sufficient to give a 0.1 N HCl solution. NaCl was then added to make 9 ml. of a 0.9% solution, which was autoclaved as in (*a*) above.

(*c*) One animal: NaOH added to 38.8 μc of Y^{91} chloride in a quantity sufficient to give a 0.1 N HCl solution. NaCl was then added to make 3 ml. of a 0.9% solution, which was autoclaved as in (*a*) above.

(*d*) Two animals: 8.4γ and 0.01γ of stable yttrium chloride in 0.1 N HCl solution was taken and NaCl added in a quantity sufficient to make 5 ml. of a 0.9% solution. This was then autoclaved at 17-lb. pressure for 60 minutes.

The distilled water used was produced by the same distillation apparatus as that in the study reported above.

Check cultures for bacterial and fungi contaminates were made of all solutions and materials used. Animals were killed 36 days after treatment, in the same manner as the animals in the first study. No clinical symptoms were recorded. Tissues were obtained immediately, fixed in 10% formalin, sectioned, and stained with hematoxylin and eosin.

Histopathology

There was no difference in the microscopic lesions, or its distribution, in the sheep comprising the second animal series, irrespective of the substance with which they were dosed. No gross lesions were observed. Solution of stable yttrium, solutions of radioyttrium, and isotonic NaCl solutions were all capable of producing lesions identical with those seen in the original group of animals. No central nervous system changes were noted, with one exception. An ovine fetus, in addition to lymphoid hyperplasia, had several cerebral foci of lymphocytic cells. Because there was only one common agent in this series, i. e., distilled water, pyrogens were considered a very likely possibility for an etiological agent.

C. PYROGEN STUDIES WITH RABBITS

Method

In this study 12 rabbits, in two groups of 6 each, were used to test the suspected vehicle for pyrogens by injecting, intravenously, control and test animals.‡ In the first test three rabbits were injected with 10 ml. per kilogram of body weight of the suspected distilled water, and three rabbits were injected with commercial pyrogen-free distilled water. Check cultures were made as before. A second duplicate test was made with the same grouping. The rabbits were healthy, laboratory-raised animals of mixed breeding. All were over 4 lb. (2 kg.) in weight and had never been used for any test before. Temperature ranges for the animals were established two and three days before the injection day, and from before the pretest temperatures until after the temperatures following injection were completed the rabbits were housed separately. Environmental temperatures were held at 27 C. Rectal thermometers were calibrated prior to use. Food was withheld from one hour before the test until all tests were completed.

TABLE 2—*Response of Rabbits to a Suspected Bacterial Pyrogen Administered Intravenously*

Animal	Wt., Kg.	Treatment	Temperature Change
		Group A	
12	3.47	34.7 ml. pyrogen-free H_2O	+ 1.2 F
43	1.97	19.7 ml. pyrogen-free H_2O	+ 1.6 F
0	3.27	32.7 ml. pyrogen-free H_2O	+ 1.8 F
27	3.20	32.0 ml. test H_2O	+ 3.6 F
90	3.68	36.80 ml. test H_2O	+ 2.4 F
21	3.4	34.0 ml. test H_2O	+ 4.2 F
		Group B	
4	3.50	35.0 ml. pyrogen-free H_2O	+ 2.6 F
5	3.10	31.0 ml. pyrogen-free H_2O	— 0.6 F
6	3.50	35.0 ml. pyrogen-free H_2O	— 0.5 F
7	3.50	35.0 ml. test H_2O	+ 3.2 F
8	3.40	34.0 ml. test H_2O	+ 2.6 F
9	2.75	27.5 ml. test H_2O	+ 3.6 F

Results

The results of the tests for the suspected pyrogenic activity of the distilled water are given in Table 2. The temperature rise in those animals given the test water intravenously was significantly higher than that in the controls.

Only rabbits from Group A came to autopsy. These were done 14 days after treatment. Tissues were fixed in 10% formalin immediately after being killed and were later sectioned and stained with hematoxylin and eosin. There was no gross change noted, except for a moderate pulmonary congestion

‡ Method suggested by Lieut. Col. T. C. Jones, V. C., Armed Forces Institute of Pathology, Washington 25, D. C.

Fig. 6.—Exudate about portal triad and along the interlobular septa in a rabbit given 10 ml/kg. of suspected pyrogen-containing distilled water. This rabbit had a temperature rise 3 hr. after the intravenous injection. It was killed 14 days after treatment.

in those given the suspected pyrogen-containing water. On microscopic examination lesions similar to that seen in the cattle and sheep were found in rabbits given the test water (Figs. 6 and 7), with the exception that they were not present in the intestine or central nervous system. It was assumed that the agent causing the elevation in temperature was also that causing the granulomatous lesion.

COMMENT

It seemed more than coincidental that the granulomatous lesion observed should have occurred in all of the animals here reported. The evidence was clear that the vehicle used in these animals, i. e., distilled water, was pyrogenic. Whenever the vehicle was injected into the blood stream of animals, there developed a widely dispersed granulomatosis associated with the small vascular network. Once the lesion was established, recovery was slow. One steer (Y-2) after 325 days still had well-defined lesions.

In two cases the etiologic agent was able to pass the placental barrier and establish foci in the heart, liver, brain, and lymphoid tissues of the fetus. The variation in the severity of the lesions was great. It ranged from only occasional foci to an extreme involvement of all vital organs, which finally necessitated destruction. So far as is known, there was no correlation between the magnitude of the response of the animal and the volume of pyrogen-containing distilled water administered. There was a general correlation between the soft organ retention of radioyttrium and the location of the pathologic lesion. An exception to this was found in the bone marrow and the pituitary, where there was high retention but no detectable lesion, and the brain, where there were occasional lesions but only minimal retention.[2] It was considered most unusual that the myocardium retained a relatively large amount of radioyttrium and was also a frequent site of the granulomatous lesions.

So far as is known, there has been no report of microscopic lesions due to intra-

Fig. 7.—The central vein of a rabbit which received 10 ml/kg. of test distilled water and was killed 14 days later. There was a postinjection temperature rise.

venously administered pyrogens since the term was first used by Seibert and her associates.[5] It is possible that changes similar to those seen in our study have been erroneously ascribed to other etiologic agents. The findings here reported should alert those who use untested vehicles, particularly distilled water, for the intravenous administration of therapeutic or other agents in experimental procedures.

Mr. Robert Sellers prepared the histologic materials. Prof. I. B. Whitney made the radiochemical solutions. Messrs. John J. Lane and Joseph C. Schoolar made the rabbit assay for pyrogens. The radioyttrium was obtained from the Oak Ridge National Laboratories.

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4. Hansard, S. L.; Comar, C. L., and Plumlee, M. P.: Radioisotope Procedures with Farm Animals: II. Quantitative Administration and Blood-Sampling, Nucleonics **9**:38-45, 1951.

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News and Comment

DEATHS

Dr. Monroe J. Schlesinger.—Dr. Monroe J. Schlesinger, pathologist-in-chief of the Beth Israel Hospital, Boston, and of the Department of Pathology of Harvard University Medical School, died on Jan. 30, 1955. According to a newspaper account, he had a heart attack while attending a meeting of the New England Society of Pathology.

Dr. John Erik Kraus.—Dr. John Erik Kraus, for many years pathologist and director of laboratories at St. Francis Hospital, Peoria, Ill., died Jan. 17, 1955, at the age of 67. Dr. Kraus was a member of the American Association of Pathologists and Bacteriologists and of the College of American Pathologists, and he had served as professor of pathology at the University of Prague before coming to the United States.

GENERAL NEWS

Lecture on Atherosclerosis.—Dr. G. Lyman Duff, Strathcona Professor of Pathology and dean of the McGill University Faculty of Medicine, gave the Dr. Jack W. Kolson Memorial Lecture at the Sinai Hospital, Baltimore, on March 24, 1955. His subject was "The Pathogenesis of Atherosclerosis and Current Concepts of Treatment."

Lecture by Dr. F. Peyton Rous.—Dr. F. Peyton Rous delivered the annual Jean Redman Oliver lecture at the State University of New York College of Medicine on Feb. 24, 1955. His subject was "Cancer Causation as Viewed Today."

Middle Eastern Pathologists Meet.—The annual meeting of the Middle Eastern Region of the College of American Pathologists was held in Philadelphia on March 10. The meeting was cosponsored by the Philadelphia Pathology Society and the section on clinical pathology of the Philadelphia County Medical Society.

Lecture by Dr. William Boyd.—Dr. William Boyd of Toronto was among the guest speakers at the 18th annual meeting of the New Orleans Graduate Medical Assembly.

PERSONAL

Appointments.—Dr. John W. Hall has been made professor of pathology at the College of Medicine of New York University.

Dr. A. James Gill, formerly professor of pathology at Southwestern Medical School, has been appointed dean.

Dr. John G. Kidd of Cornell University Medical College has been elected to the board of scientific directors of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Scientific Exhibits

NEOPLASMS OF THE THYROID

Classification, Morphology, and Treatment

JOHN B. HAZARD, M.D. GEORGE CRILE JR., M.D. ROBERT S. DINSMORE, M.D.
W. A. HAWK, M.D. AND REX KENYON, M.D.
CLEVELAND

FOREWORD

THE EXHIBIT is concerned with the analysis of 2468 discrete adenomas of the thyroid and 400 malignant tumors of the thyroid. The purposes are to present a clinicopathologic classification of the material, to summarize the distinctive pathologic and clinical aspects, and to indicate certain trends in prognosis and therapy in the malignant group.

From The Cleveland Clinic Foundation and The Frank E. Bunts Educational Institute, Cleveland.

Shown as a scientific exhibit of the Section on Pathology and Physiology at the 103rd Annual Meeting of the American Medical Association, San Francisco, June 21-25, 1954.

Head, Department of Pathology, Cleveland Clinic (Dr. Hazard); Staff, Department of General Surgery, Cleveland Clinic (Dr. Crile); Head, Department of Surgery, Cleveland Clinic (Dr. Dinsmore); Fellow, Department of Pathology, Cleveland Clinic (Dr. Hawk); Formerly Fellow, Department of Pathology, Cleveland Clinic, now at Barnes Hospital, St. Louis (Dr. Kenyon).

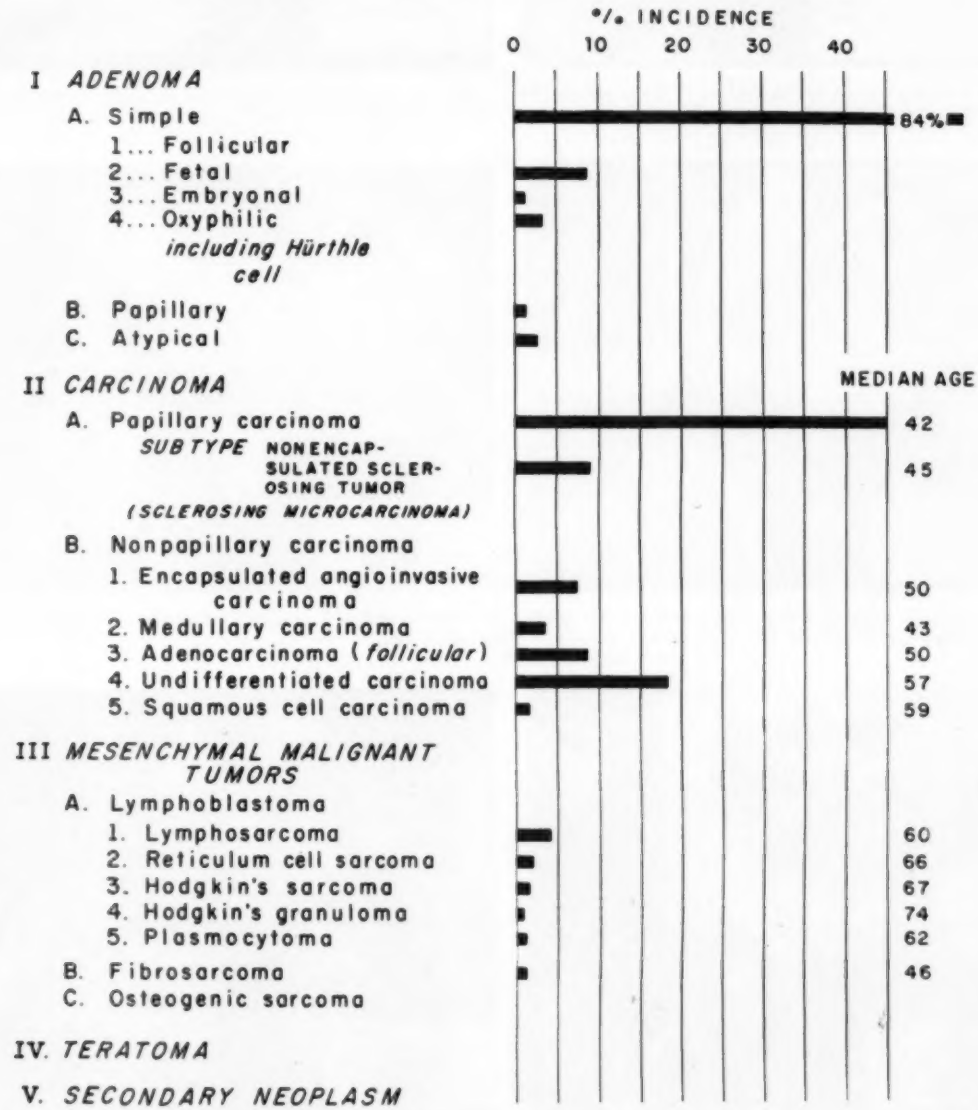
The malignant tumors of the thyroid basically are separated into two major groups, papillary and nonpapillary; further divisions are based on clinicopathologic characteristics. The papillary group comprised the largest number of malignant tumors. Frequent in this group was a variant that was predominantly of follicular structure, but with the behavior of a truly papillary neoplasm. Among nonpapillary carcinomas, the descending order of frequency was undifferentiated carcinoma, encapsulated angioinvasive carcinoma, adenocarcinoma (follicular carcinoma), and medullary carcinoma. The medullary carcinoma, of sheet-like structure with uniform cell type, previously has not been described as an explicit group of nonpapillary neoplasms. The carcinomas formed by Hürthle cells are not separately classified, as they do not appear to form a distinctive

PANEL II—ADENOMA

clinicopathologic group, the clinical behavior being indicated by the type of neoplasm to which the tumor belongs and not by the staining property of the cells. The Hürthle-cell carcinomas occurred most commonly as encapsulated angioinvasive carcinoma and adenocarcinoma (follicular carcinoma). The carcinomas of undifferentiated type appeared often as more than one subtype in a single tumor; frequent combinations were of solid, spindle-cell, and giant-cell variants. The clinicopathologic aspects of the various malignant tumors are indicated in the appropriate panels.

PANEL I

CLASSIFICATION OF THYROID NEOPLASMS



PANEL II—ADENOMA

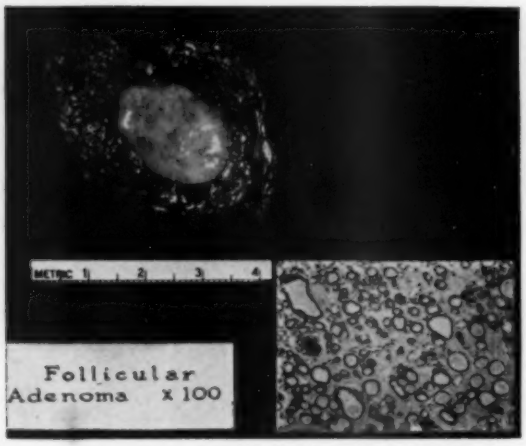


Fig. 1.—Bulging, glistening surface. Typical follicles in edematous stroma.

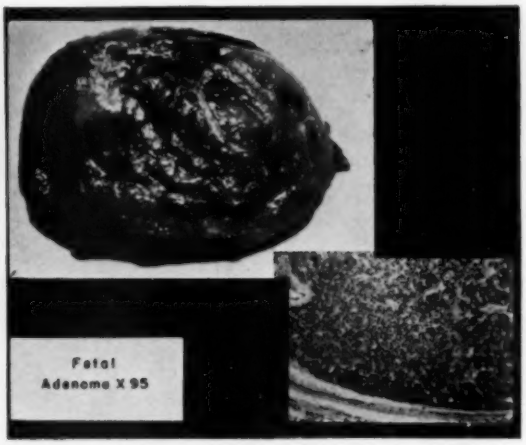


Fig. 2.—Slightly bulging surface, partially opaque. Small closed follicles, closely packed in peripheral zone.

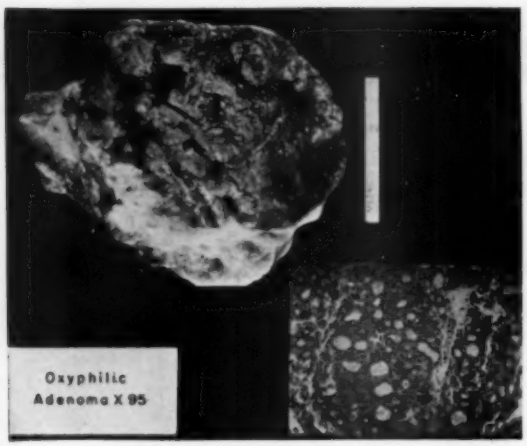


Fig. 3.—Dull surface; tumor was a brown color. Cells were oxyphilic, of large size (Hürthle cells).

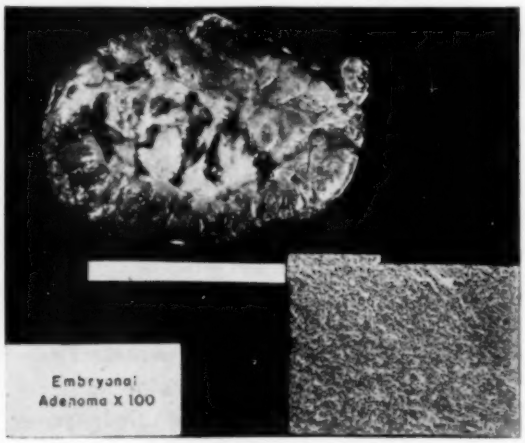


Fig. 4.—Bulging, opaque surface. Closely packed cords of small cells.

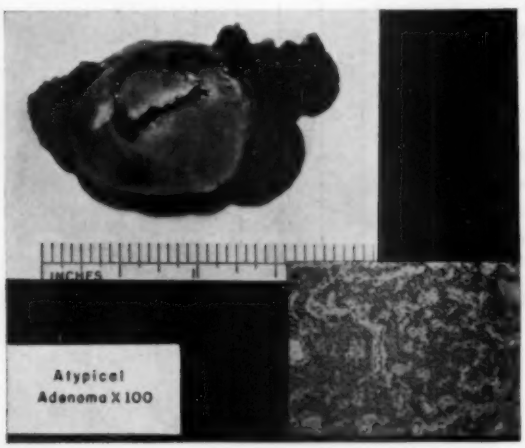


Fig. 5.—Opaque, flat surface; gray-white color. Irregular cords and sheets of cells with fibrous stroma.

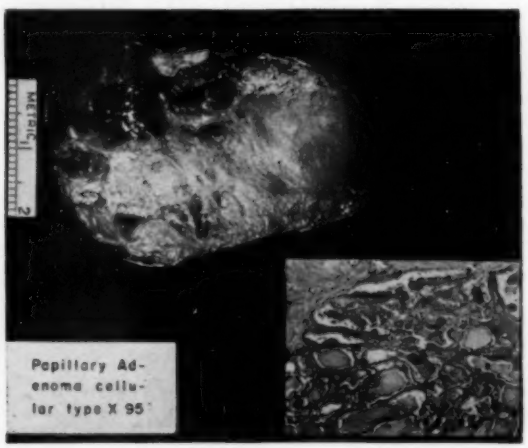


Fig. 6.—Slightly bulging, dull, granular surface. Papillary configuration with follicular areas. Capsule was intact in multiple blocks.

PANEL III—PAPILLARY CARCINOMA

CLINICOPATHOLOGIC ASPECTS: *Age range*: 3.5 to 77 years; median, 41.5 years. *Sex*: F/M: 3/1. Tends to metastasize to regional lymph nodes. *Several forms*: (*a*) palpable thyroid tumor with palpable cervical metastases; (*b*) palpable thyroid tumor without cervical metastases; (*c*) cervical metastases without palpable thyroid tumor; (*d*) massive thyroid tumor (fused with proximal cervical metastases). Neoplasm of hard consistence. *Mortality* from cancer in 153 cases: 16 deaths (10.5%); 10, within 5 years of diagnosis; 2, more than 5 years after diagnosis; 4, more than 10 years after diagnosis. Average age in fatal group: 56 years, first visit; 61 years, at death. Status of cancer at death: in 12, massive local lesions; in 8, metastases to lungs, more rarely to bone and liver. Reasons for recurrence or therapeutic failure: incomplete primary operation; inoperable lesion.

Primary tumor (142 cases). Solitary, 81.7%; multicentric, 18.5% (both lobes involved, 9.9%). Regional lymph nodes involved at primary operation, 54%: unilateral, 41%; bilateral, 13%. Histologic findings in fatal cases: markedly papilliferous, 10; follicular, 4; poorly differentiated, 2.

THERAPY: Dependent on gross character of tumor.

Solitary without involvement of regional lymph nodes: Complete removal of affected lobe and isthmus. Isthmus examined histologically at surgery for multicentricity. If multicentricity absent, no further surgery.

Solitary with involvement of regional lymph nodes: Treatment as above but with excision of involved lymph node groups en bloc with preservation of sternocleidomastoid muscle.

Multicentric lesions: Total thyroidectomy with preservation of at least two parathyroids. Bilateral excision of involved lymph node groups, if present.

Fig. 1.—Irregular tumor nodule with white scar. Tissue was of light ocher color, firm, tough; not grossly papillary.

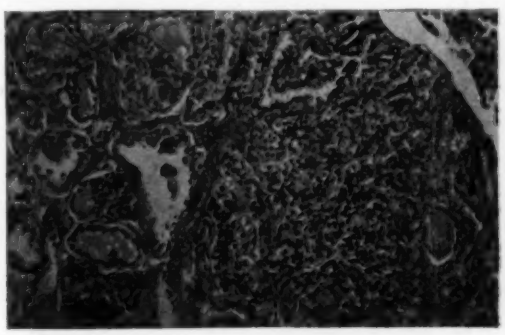


Fig. 2.—Papilliferous area in upper right center; elsewhere closely packed cells, follicle formation; irregular fibrous stromal bands.

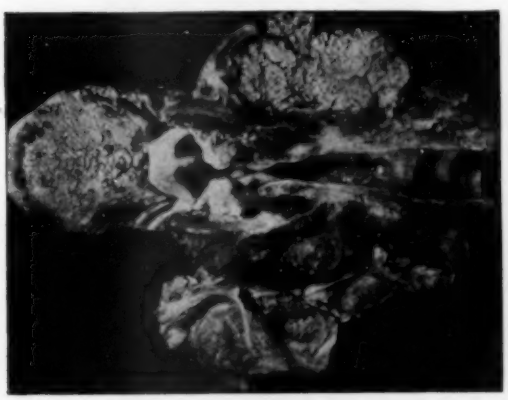


Fig. 3.—Massive papilliferous tumor mass replacing most of thyroid; metastasis to regional lymph nodes; invasion of trachea.

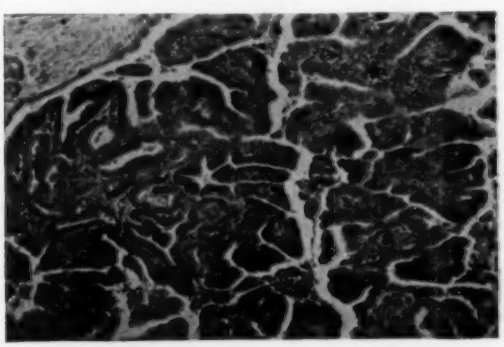


Fig. 4.—Markedly papilliferous structure; cells of tall columnar type.

PANEL IV—PAPILLARY CARCINOMA

Figure 1

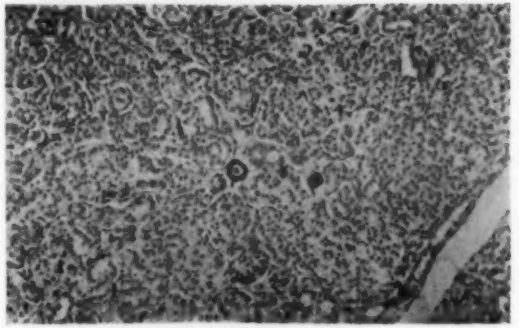


Figure 2

Figure 3

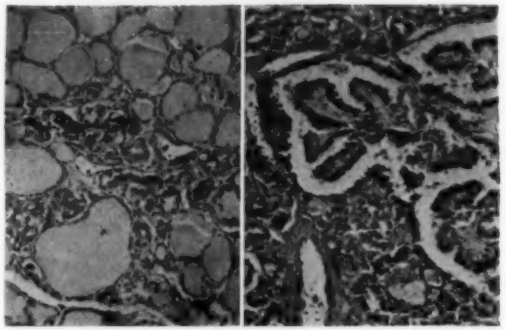


Figure 4

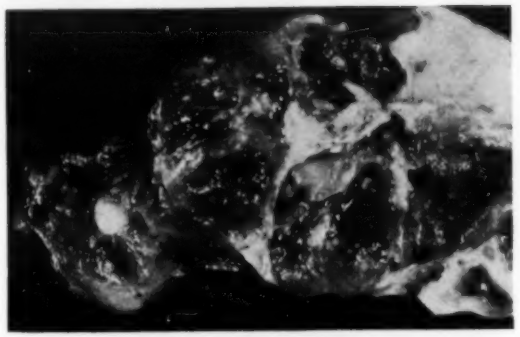


Figure 5

Fig. 1.—Circumscribed, single nodule.

Fig. 2.—Papillary configuration not evident; psammoma calcification suggests true nature.

Fig. 3.—Pseudoencapsulate, mostly white, tumor nodule.

Fig. 4.—*Left:* Chiefly follicular. *Right:* Typically papillary area in same tumor.

Fig. 5.—Nonencapsulated sclerosing tumor (sclerosing microcarcinoma). Small white scar-like focus on left.

PANEL IV—PAPILLARY CARCINOMA, Contd.

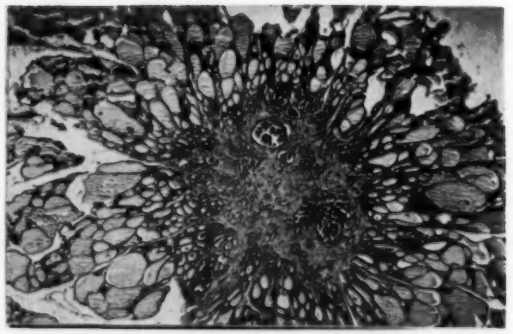


Figure 6

Figure 7

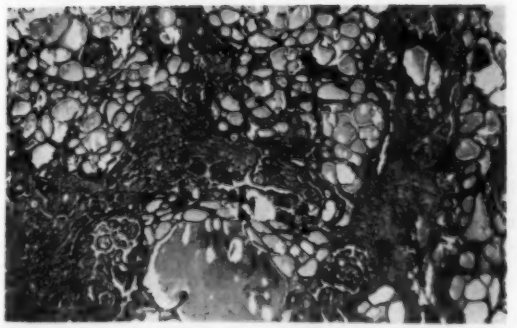


Figure 8

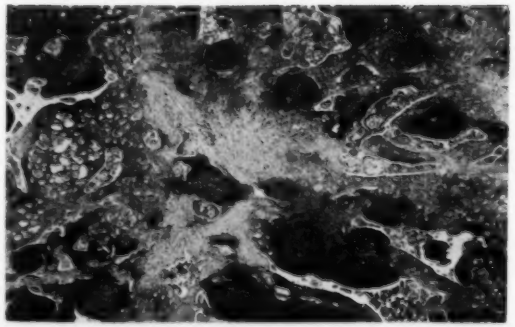


Figure 9

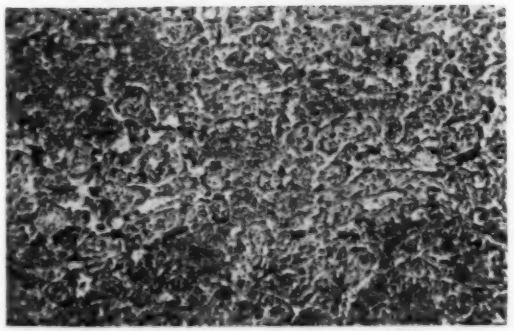


Figure 10

Fig. 6.—Nonencapsulated sclerosing tumor. Marked fibrosis, focally papillary, puckered appearance.

Fig. 7.—Small, white tumor, isthmus; extensive lymph node metastasis.

Fig. 8.—Small, papillary carcinoma; no sclerosis.

Fig. 9.—In lymphoid thyroiditis.

Fig. 10.—Closely packed papillae; cells were poorly differentiated.

PANEL V—NONPAPILLARY CARCINOMA

ENCAPSULATED ANGIOINVASIVE

CLINICOPATHOLOGIC ASPECTS: *Age*: median, 50 years. *Sex*: F/M: 3/2. Progressive, usually unilateral thyroid enlargement. *Course*: Of 22 patients traced five years or more following therapy by subtotal thyroidectomy or partial lobectomy, 68% had recurrence and/or metastases; the mortality rate in this group was 20%. The over-all mortality rate was 12.5%.

Encapsulate, 2 to 14 cm.; firm, rubbery, opaque, gray to gray-pink, friable; flat cut-surface. Occasionally areas of hemorrhage, necrosis, fibrosis, cyst formation. Rarely, gross invasion of blood vessels; in 9 of 10 cases angioinvasion is microscopic. Characteristically, invasion of capsular vessels by endothelium-covered tumor masses. No lymph node metastases found.

THERAPY: Total lobectomy. In inoperable recurrence with colloid, I^{131}.

Fig. 1.—Encapsulated; gray-white tissue.

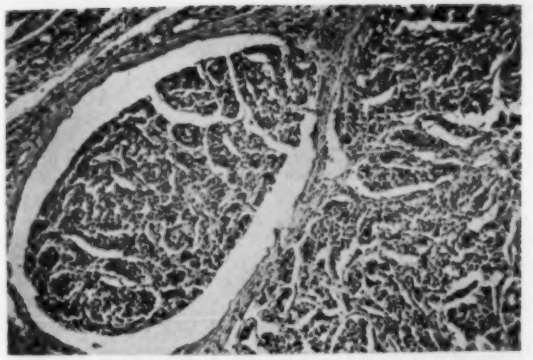


Fig. 2.—Intravascular tumor mass, endothelial covered.

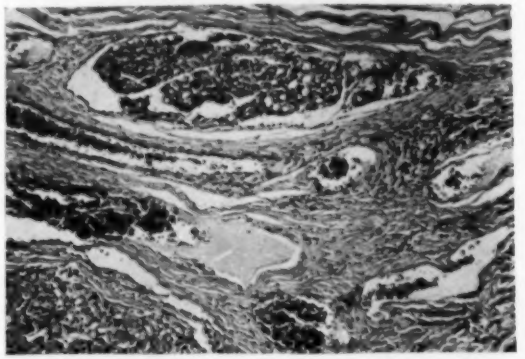


Fig. 3.—Intravascular tumor mass, outer aspect of capsule.

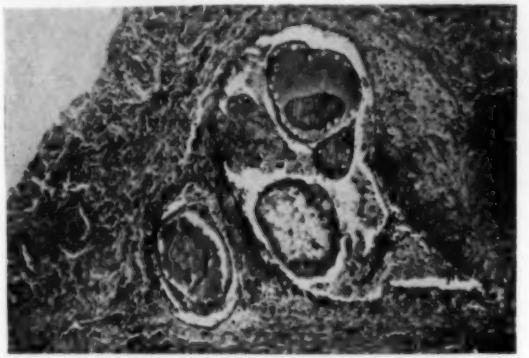


Fig. 4.—Thrombus about tumor elements.

PANEL V—NONPAPILLARY CARCINOMA, Contd.

ADENOCARCINOMA (FOLLICULAR)

CLINICOPATHOLOGIC ASPECTS: *Age*: median, 50 years; range, 23 to 75 years. *Sex*: F/M: 3/1. Usually, hard unilateral mass; occasionally, diffuse thyroid tumor; rapid enlargement. *Mortality*: 66.7%. Prone to local recurrence.

Usually some portion of an encapsulated tumor is recognizable. In some cases the neoplasm may be diffuse. Microscopically, angioinvasion frequently is seen. Rarely, lymph node involvement.

THERAPY: Radical resection; excision lymph nodes when involved. I^{131} may be used if colloid present in recurrent tumor or metastases.

Fig. 5.—Partly encapsulate; invasive at top.

Fig. 6.—Diffuse thyroidal involvement.

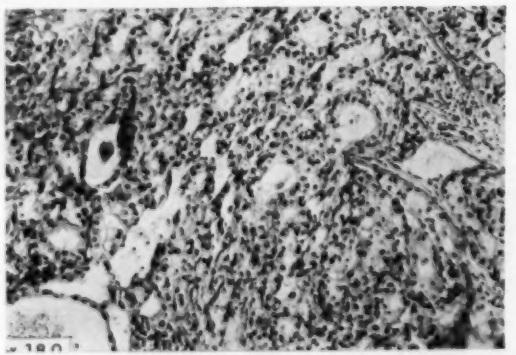


Fig. 7.—Well-differentiated, thyroid follicles.

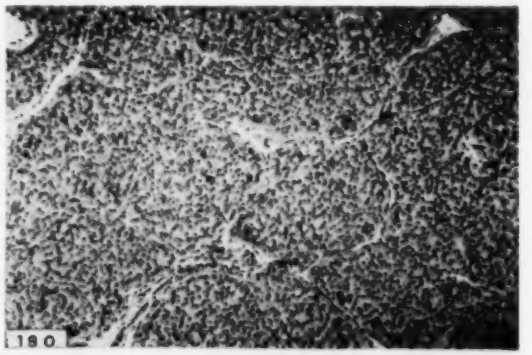


Fig. 8.—Closely packed, chiefly closed, follicles.

PANEL VI—NONPAPILLARY CARCINOMA

UNDIFFERENTIATED

CLINICOPATHOLOGICAL ASPECTS: *Age*: median, 57 years; almost never less than 40 years. *Sex*: F/M: 2/1. Often large, hard mass firmly fixed to tissues of neck. Short course: rapid enlargement of thyroid, pain, occasionally respiratory difficulty. *Mortality*: 95% of group dead; majority of patients die within six month after diagnosis.

Diffuse, or occasionally some semblance of capsular outline. Grossly infiltrating and composed of gray-white to yellow-gray tissue, hard to firm. Lymph node metastases may occur. Gross involvement of blood vessels may occur. Microscopically, several subtypes: solid, spindle cell, giant cell, small cell, scirrhous, hypernephroid, oxyphilic; first three commonest, usually mixed. Scirrhous subtype characterized by absence of any capsular outline, uniformly dense, fibrous.

THERAPY: None curative; roentgen therapy may be palliative.

Fig. 1.—Irregular, bulging tumor mass; fibrous zones partly infiltrated by tumor, suggested preceding Riedel's struma.

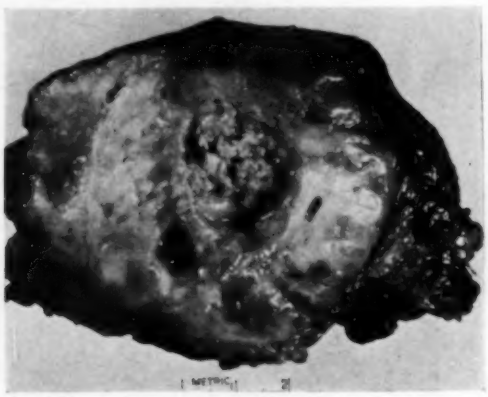


Fig. 2.—Partly encapsulated; invasive on left.

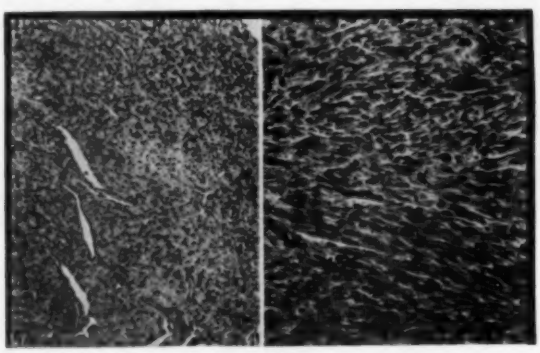


Fig. 3.—*Left:* Solid. *Right:* Spindle cell.

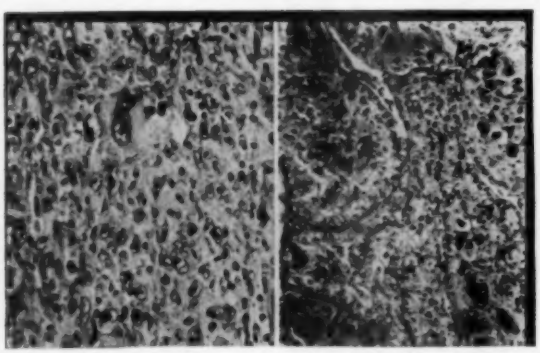


Fig. 4.—*Left:* Giant cell. *Right:* Hypernephroid.

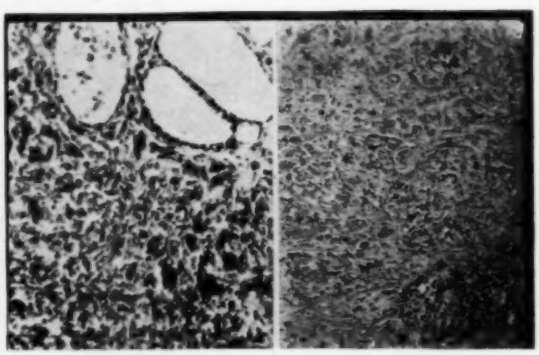


Fig. 5.—*Left:* Small cell, with osteoclast-like giant cells. *Right:* Scirrhous.

PANEL VII—NONPAPILLARY CARCINOMA: RARE TYPES

MEDULLARY

CLINICOPATHOLOGIC ASPECTS: *Age*: median, 42 years. *Sex*: nearly equal distribution. Hard tumor mass in thyroid, frequently with palpable regional lymph nodes. *Mortality*: Of 12 patients, 3 died (mortality, 25%).; local recurrence, metastases to regional lymph nodes, lungs, liver, and bone.

Primary tumor: frequently circumscribed, but not encapsulated; may invade thyroid. Degree of malignancy probably between that of papillary carcinoma and that of adenocarcinoma.

THERAPY: Radical lobectomy or total thyroidectomy and resection of involved lymph node groups.

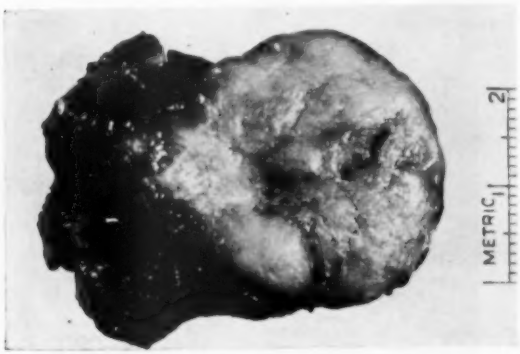


Fig. 1.—Circumscribed tumor; grossly similar to papillary carcinoma.

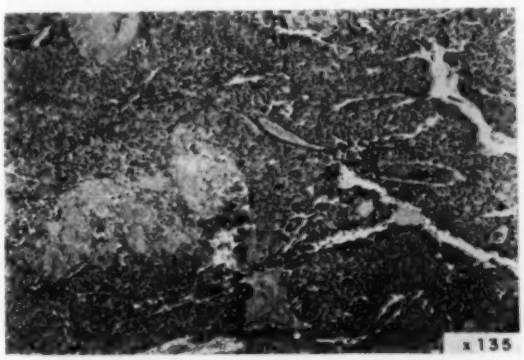


Fig. 2.—Sheets of cells; fibrous stroma with amyloid-like areas. Nuclei uniform; cells never show mitotic activity or dedifferentiation of the solid variant of undifferentiated carcinoma.

SQUAMOUS CELL

CLINICOPATHOLOGIC ASPECTS: *Age*: median, 59 years. *Sex*: 2 women, 1 man. Hard mass tending to be fixed; indistinguishable grossly from undifferentiated carcinoma. *Prognosis*: poor.

Infiltrating, hard, white or yellow-white mass.

THERAPY: None curative; roentgen therapy may be palliative.

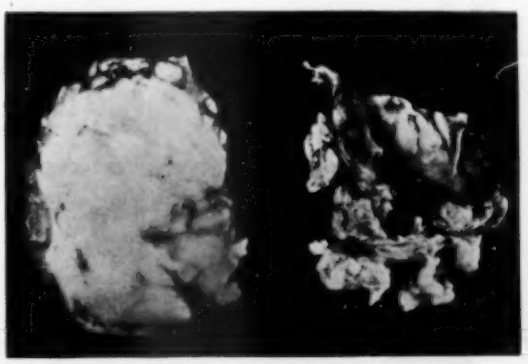


Fig. 3.—Bulky, white tumor mass replacing much of thyroid.

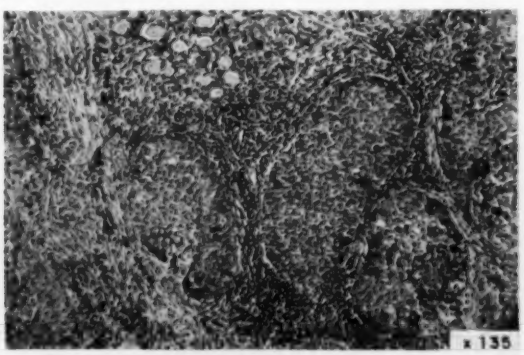


Fig. 4.—Islands of squamous cell carcinoma infiltrating marginal thyroid.

LYMPHOBLASTOMA

CLINICOPATHOLOGIC ASPECTS:

Type	Incidence, %	Median Age (Yr.)	Sex F	Sex M
Lymphosarcoma	4.0	60	13	3
Reticulum cell sarcoma..	1.3	66	4	1
Hodgkin's sarcoma.....	1.0	67	3	1
Hodgkin's granuloma...	0.2	74	0	1
Plasmocytoma	0.5	62	0	2

Characteristically, rapidly enlarging thyroid mass with fixation. *Mortality*: 26 of 28 patients dead within 10 years after diagnosis (mortality, 92.8%). Each of two patients having plasmocytoma survived 10 years or longer; one with, and one without, recurrence.

Fleshy, soft, gray-white, diffusely infiltrating, replacing much of thyroid and frequently infiltrating adjoining tissues. Occasionally, persistent oxyphilic thyroid follicles found in infiltrating tumor mass.

THERAPY: Roentgen radiation; when disseminated, treat as lymphoblastoma of other sites.

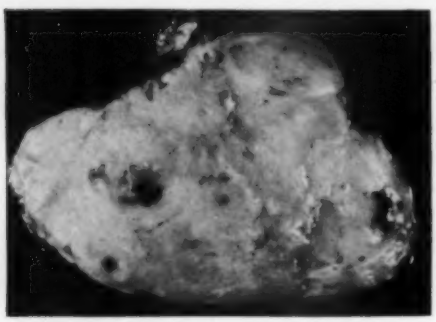


Fig. 1.—Lymphosarcoma.

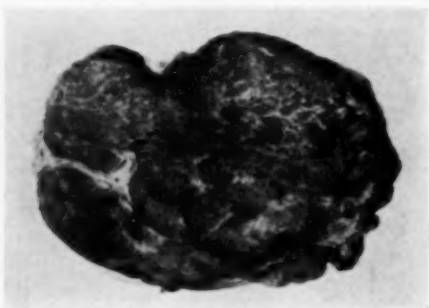


Fig. 5.—Plasmocytoma.

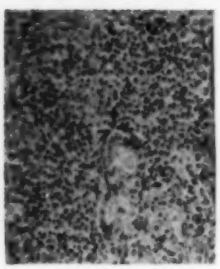


Fig. 2.—Lymphosarcoma. Diffuse infiltration by lymphocytes; few remaining follicles.

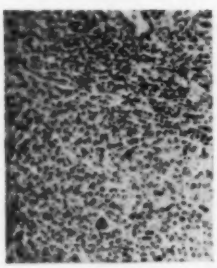


Fig. 3.—Hodgkin's granuloma.

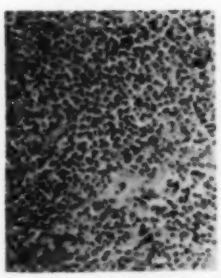


Fig. 6.— Plasmocytoma. Diffuse infiltration by plasma cells.

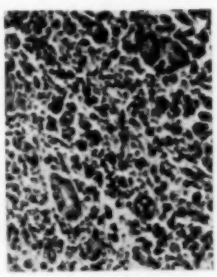


Fig. 7.—Hodgkin's sarcoma.

Fig. 4.—Hodgkin's granuloma; tissue unusually soft.

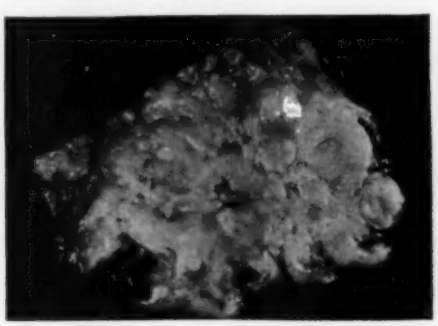


Fig. 8.—Hodgkin's sarcoma.

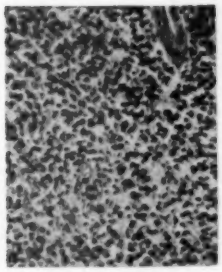


Fig. 9.—Reticulum cell sarcoma.

FIBROSARCOMA

CLINICOPATHOLOGIC ASPECTS: *Age*: average, 46 years. *Sex*: both women. Rapidly enlarging thyroid mass. *Mortality*: One patient died within one year; other patient died within four years (unrelated cause).

Bulky, hard, white, infiltrating tumor mass.

THERAPY: Surgical excision when possible.

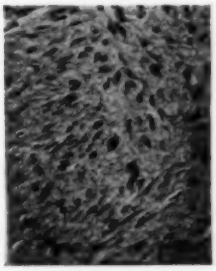


Fig. 11.—Fibrosarcoma; well-differentiated fibroblasts.

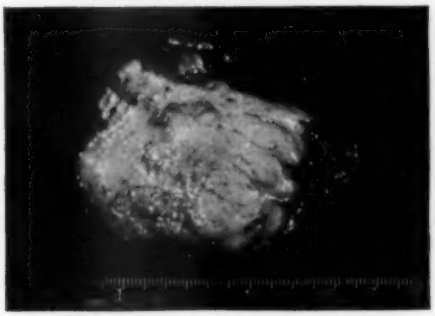


Fig. 10.—Reticulum cell sarcoma.

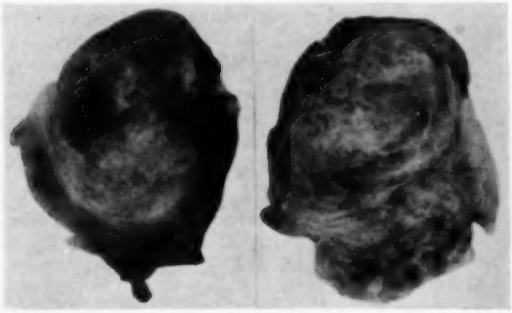


Fig. 12.—Fibrosarcoma, involves most of lobe.

Books

Connective Tissue in Health and Disease. Edited by G. Asboe-Hansen, M.D. Price, $7.50. Pp. 321. Ejnar Munksgaards Forlag, Nørregade 6, Copenhagen, Denmark, 1954.

This book is composed of a series of contributions by many eminent investigators in the field. The material included covers the whole range of current activities in the area. Starting with the normal embryology and morphology of the connective tissue, it surveys the histochemistry, chemistry, and metabolism of mucopolysacharides, and the sulfate exchange, spreading reaction, and inhibition of hyaluronidase. Following this are discussed various aspects of hormonal influence on connective tissue and infection and the functional relationship of connnective tissue to infection and to cancer. Arteriosclerosis, collagen diseases in general, arthritis, mesenchymal aspects of diseases of the eye and the skin, and fibrosis due to injury are likewise discussed in their relationship to connective tissue. These subjects are presented by such authors as Robb-Smith, McManus, Karl Meyer, Dorfman, Duran-Reynals, Angevine, Sprunt, Simpson, Rinehart, Ragan, Klemperer, Asboe-Hansen, and others.

A great amount of new and original material is included in this volume, along with much which has been said before. To anyone interested in the general field of connective tissue function and disease, this is a worthwhile acquisition. It also gives a good survey of many areas of this popular field for those interested in viewing the current panorama.

Relation of Fibroadenomatosis (Chronic Mastitis) to Cancer of the Breast. By William Kiaer. Price, 30 Danish kroner. Pp. 149, with 71 figures. Ejnar Munksgaards Forlag, Nørregade 6, Copenhagen, 1954.

This study concerns the problem whether women with chronic mastitis (or fibroadenomatosis) develop more breast cancer than other women. The histological material consisted of 700 clinically normal breasts removed at autopsy from 350 women, 378 breasts removed for cancer, and 334 cases of clinically manifest fibroadenomatosis. Latent fibroadenomatosis was about twice as common in cancerous breasts as in the controls. A long-term follow-up of 321 patients with histologically proved fibroadenomatosis showed that they developed breast cancer more frequently than expected according to normal morbidity; the association was most striking in high-grade fibroadenomatosis. Many other conclusions are made. This work is a well-illustrated, carefully documented study of a perplexing problem.

Pathology. By W. A. D. Anderson. Price, $16.00. Pp. 1,393, with 1,241 illustrations, 10 in color. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1953.

A textbook of pathology suitable for beginning students and for the postgraduate physicians is a difficult undertaking. This textbook, the joint effort of many eminent American pathologists, has accomplished the dual role admirably. The first five chapters cover the basic reaction patterns to injury, usually taught in a general introductory course. Chapters 6 through 17 present a detailed coverage of the pathology associated with infections and metabolic diseases of known etiology. Current trends in medicine make the sections on physical, chemical, and radiation injury particularly useful and informative. A general introduction to neoplasms in Chapter 18 is presented. The remainder of the volume is occupied with presentation of pathology by systems with two exceptions, a chapter on the pathology associated with diabetes mellitus, and the final chapter on "Heredity and Constitution in Disease."

The book is well printed in the current two-column style. The pictures and charts are clear and, for the most part, well chosen. Bibliographies are extensive and usually quite up to date. The book is to be highly recommended for students, for practitioners, and for the library of any modern pathologist. It offers, in an easily available source, many facts, general surveys, and entrance into the literature of disease not to be had in any other modern American source.

As with any undertaking of this scope, there are shortcomings, and not all areas are covered equally well; but, on the whole, it is an excellent work.

Peripheral Circulation in Man: A Ciba Foundation Symposium. By G. E. W. Wolstenholme, Editor for Ciba Foundation, assisted by Jessie S. Freeman and Joan Etherington. Price, $6.00. Pages, 219, with 72 illustrations. Little, Brown & Company, 34 Beacon St., Boston 6, 1954.

The material presented in this book will be of interest to all concerned with the nature of, control of, and pathologic changes in the peripheral circulation. It represents the reflections and the experimental observations of a group of eminent specialists in the field of vascular function. The topics covered include methods of studying blood flow with critical reflections and comments by the experts involved, observations on the effects of heat and cold, the action of epinephrine (Adrenalin) and arterenol (Noradrenalin), neurohistology, and reflex control of circulation. Also discussed are certain pathologic conditions, such as Raynaud's phenomenon and its relationship to cold agglutinins in the serum.

The structure of the book is that common to most symposia, with presentation of individual work and extensive and free discussion of the techniques, results, and implications. The first portion of the discussion deals with the means of observing and measuring blood flow and the validity of the methods. The latter parts deal with the physiologic and pathologic alterations observed with the methods. This juxtaposition of discussion of method and of results by the experts provides an opportunity for evaluating our current knowledge of peripheral vascular phenomena not offered in many compilations.

Since the circulation enters importantly into almost all, if not all, pathologic processes, this book, presenting an essence of modern thought in the area of vascular structure and function, will be of interest to pathologists. It will be of most interest to those considering the role of the circulation in inflammation and related situations.

Clinical Approach to Jaundice. By Leon Schiff, M.D., Ph.D. Price, $3.75. Pp. 113, with 52 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1954.

Diagnosis of the etiology of jaundice is possible in 90% to 95% of cases by means of history and physical examination, laboratory tests, and needle biopsy. In this monograph the author considers the use and interpretation of these various procedures. The points of importance in both the history and the physical examination and their meanings are discussed in the first chapter. Laboratory tests that are of value in distinguishing different types of jaundice are pointed out in the second portion of the book. The advantages and limitations of roentgen examination are mentioned. The author has had considerable experience with needle biopsies and has included a well-written and well-illustrated chapter on this supplementary procedure. The monograph presents a concise, logical approach to the problems of jaundice from a clinical point of view. The chapters on laboratory tests and needle biopsy of the liver will be of particular interest to the pathologist.

Urology. Edited by Meredith Campbell, with 51 contributors. Price, $60. Vols. I, II, and III. Pp. 2,356, with 1,148 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1954.

This timely compilation by 51 contributors gives a well-balanced coverage of urology. The anatomy and physiology are given ample space, with an especially readable section on the rapidly developing field of tubular function. The pathology is integrated into the general discussion of each section and, while this is advantageous to the urologist, it makes the volume less desirable for the pathologist than are recent works limited to urologic pathology.

Diagnostic procedures are extensively covered, including evaluation of urologic symptoms, physical examination, analyses, estimation of renal function, excretory urography, cystoscopy, retrograde pyelography, special roentgenographic examinations, hormonal studies, as well as biopsy and special cytologic study. There are 12 chapters of surgical procedures, with many standard operative procedures presented by the men who devised them. Nonsurgical urologic diseases are presented in an appropriate form. Medical treatment includes evaluation of the latest antibiotic and chemotherapeutic drugs. Endocrinology in urology is discussed, with an adidtional section on the adrenal. The method of presentation is straightforward and is extensively illustrated. The three-volume set is well indexed, each volume containing an index to the set.

Surgical Treatment of Cancer of the Cervix. Edited by Joe V. Meigs, M.D. Price, $12.00. Pp. 462, with 205 illustrations. Grune & Stratton, Inc., 381 4th Ave., New York 16, 1954.

This volume, of 462 pages, is composed of twenty-two contributions by twenty-three authors. The five foreign communities represented are Amsterdam, by one; Stockholm, by two; Calcutta, by one; Graz, by one, and Vienna, by two. The seventeen contributors from the United States are scattered across the country. The editor, Dr. Joe V. Meigs, says in the preface: "There is no doubt that the surgical approach to the disease has aroused great interest, although there are not many five year American results available at the present time. It is the editor's hope that this volume will give the surgeon or gynecologist who wishes to perform these operations an idea of the meticulous care needed before, during and after surgery. The magnitude of the operation and the pitfalls and possible damage to vital and important organs are evident." He later adds, "If surgery is to be used at all, technics must be most carefully studied."

The introduction, with references by the editor, includes twenty-four pages. It is presented in a logical sequence including the history, a discussion of operation versus radiation, the classification of cervical cancer on an international, as well as a surgical and pathological, basis, comments upon carcinoma in situ, lists of a glossary for definition of special terms, and comments upon treatment, etiology, and diagnosis. He makes brief comments upon cervical stump cancer, recurrent cancer, and lymph node involvement and gives brief space to cervical cancer in pregnancy.

The early chapters include anatomy and blood vessels of the pelvis, lymph nodes and lymph channels, and sympathetic and parasympathetic nerves. The illustrations, drawings, and cuts are numerous and properly selected.

The discussions on the surgical approach, techniques, and therapies, with tables, pictures, drawings, and cuts, throughout the rest of the book are well balanced. The text generally is coherent and easily followed. Although the abdominal approach is well presented, the editor has included the vaginal route as well, using the Schauta operation or its modification. Ample space is allotted for "exenteration." Resections of the sigmoid and of the posterior, as well as the anterior, part of the pelvis are presented separately and with equal considerations.

The book is of a convenient size, and it is printed in a very attractive type of print on an excellent quality of paper.

Any person who does pelvic surgery or who is interested in the treatment or the diagnosis of pelvic conditions could profit by reading any one of several chapters of this book. It should be of value to every gynecologist and obstetrician and to all others interested in diseases involving the pelvis. For anyone who has not been schooled in treating cervical cancer by surgery, this book is a "must." The risks, hazards, pitfalls, and complications are many. It should not be assumed that the approaches described are the final answer for cervical carcinoma, but for the present they have a place in the therapeutic approach for the few, carefully selected patients. These procedures must be restricted to institutions with a sufficient staff, proper facilities, and sufficiently skilled surgeons to cope with this extensive, unusual major approach. The volume is recommended highly.

CORRECTION

In the article "Homogenous Renal Transplantation in Dogs: Associated Positive Coombs Test and Anemia," by Dr. E. E. Muirhead and M. Groves, in the February, 1955, issue of the Archives, the following changes should be made in the citation of references:

The footnote at the bottom of the right-hand column on page 228 should read "Reference 44."

In the fifth and the ninth paragraph of the section entitled "Comment," Reference 10 should be changed to Reference 45.

The footnote at the bottom of the right-hand column on page 229 should read "References 24 and 25."

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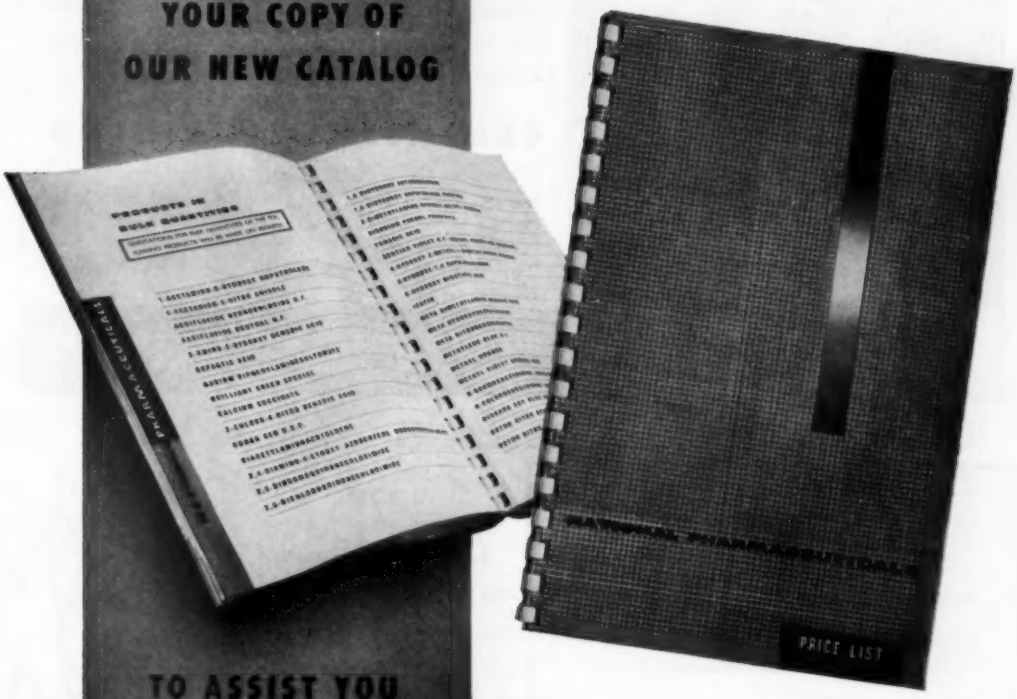

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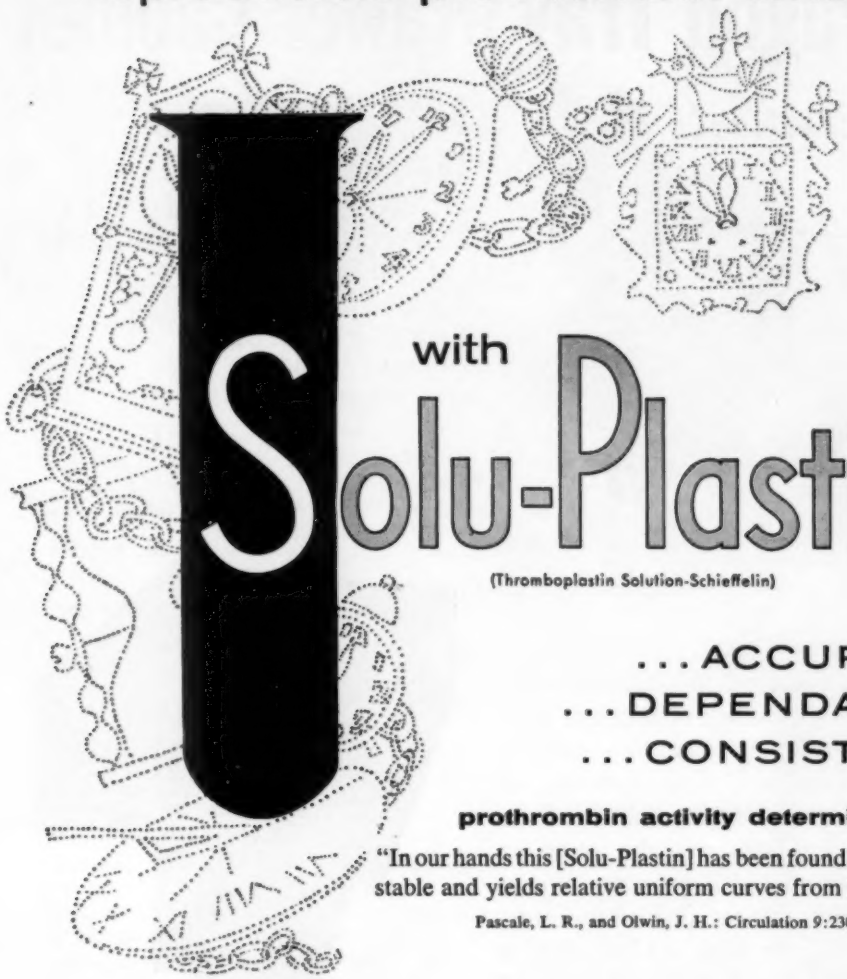

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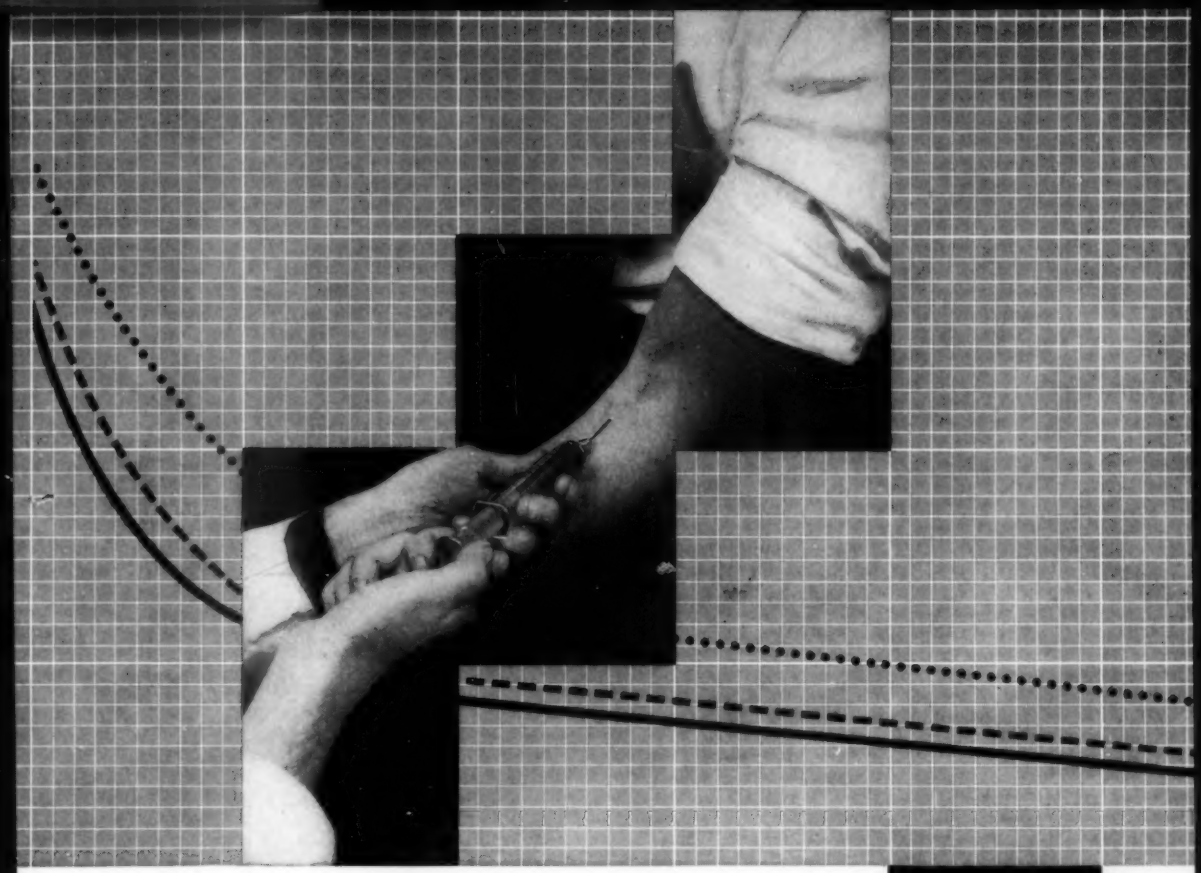

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1. Schilling, F. J.; De Natale, A., and Mottram, F. C.: Am. J. M. Sc. *222*:207 (Aug.) 1951.
2. Shapiro, S., and Weiner, M.: J. M. Soc. New Jersey *48*:1 (Jan.) 1951.
3. Shapiro, S., *et al.*: Am. Heart J. *40*:766 (Nov.) 1950.

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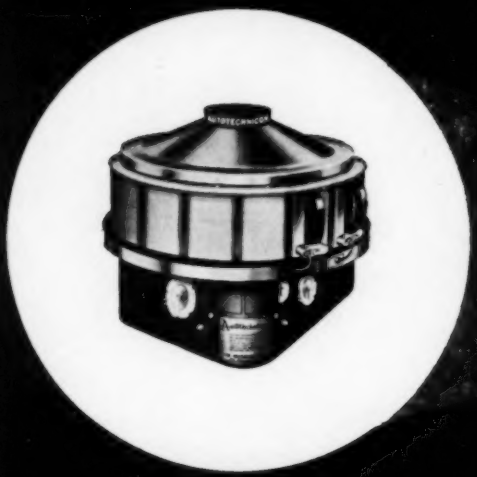

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